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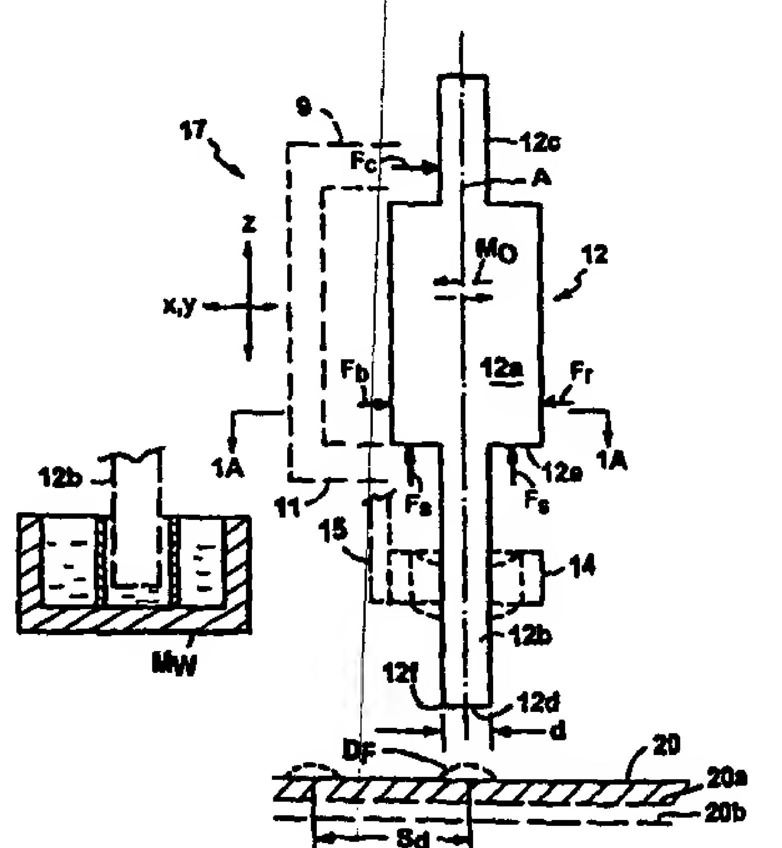
(54) Title: DEPOSITING FLUID SPECIMENS ON SUBSTRATES, RESULTING ORDERED ARRAYS, TECHNIQUES FOR ANALYSIS OF DEPOSITED ARRAYS

(57) Abstract

A deposit device (12) cooperating with a fluid source defines a precisely sized drop of fluid (DF) of small diameter on a drop carrying surface (20). Transport mechanism (17) positions the device (12) precisely over the receiving surface (20) and drive mechanism (17) moves the deposit device (12) toward and away from the surface (20). The deposit device (12) is compliant in the direction of deposition motion. When depositing, the deposit device (12) is laterally constrained to a reference position by flexure mounting. A mobile-fluid storage device resupplies the deposit device along the array. Mobile annular storage rings (14) are lowered and raised to obtain a supply of fluid, or a mobile multiwell plate is used. Cleaning mechanism, sampling plans, and array products on microscope slides and fragile or soft membranes are disclosed as are many types of fluids and uses, e.g. in biotechnology, analysis and process control.



Depositing Fluid Specimens on Substrates, Resulting Ordered Arrays, Techniques for Analysis of Deposited Arrays

Background of the Invention

The invention relates to the deposit upon substrates of small quantities of fluid in a precise manner and in arrays of desired density and consistency. The invention is useful, for instance, in carrying out reactions, in providing accurate overlays of deposits, and, in particular, in preparing microscope slides and membranes with biological materials.

The invention also relates to array products produced by the novel deposit techniques and to methods of analysis that employ the deposit techniques.

In the field of biochemistry it is desirable to accurately and efficiently deposit tens, hundreds, thousands and tens of thousands of samples of differing compositions on reaction or examination areas. Improvement in the speed of deposition, the precision of the size, shape, quantity and location of the deposits and the control over density of the deposits can lead to important advantages.

In particular, well developed biological analytical technology, and recently developed "Lab on a Chip" or "Gene Chip" techniques require creation of dense arrays of fluorescently labeled micro-organisms and DNA assays in a two dimensional field. It is desirable to place the arrays on a conventional microscope slide, and to create many such slides simultaneously in a manufacturing process.

In important applications, single stranded DNA or PNA or other biological elements in the form of fragments carrying known information are distributed onto the

differences in evaporative characteristics and in other properties.

Such large range of property variations in fluids of interest has caused great difficulties for any single type of process to operate over a wide range.

Certain processes employing photolithographic techniques have offered excellent positional accuracy of the objects and high dot density but have great limitations due to cost and due to the limited range of biological and chemical techniques and substrates that are applicable. These techniques typically construct short segments of DNA or other molecules by adding single bases, one at a time.

Certain other processes for forming arrays of dots of biological material have utilized piezo micro cylinders to aspirate and jet small volumes of fluid containing the material while others have used processes akin to a fountain pen, comprising a "quill" deposition tool. An assemblage of quills suck up a desired amount of fluid and by tapping a quill upon the receiving substrate, the meniscus holding the fluid in the gap of the quill breaks, due to inertia of the fluid within the suddenly stopped tool, so that a drop of fluid is effectively propelled from inside the quill to the impacted surface.

The development of such techniques has occurred against the background of the quite old technique for forming much larger deposits, of transferring a portion of fluid by a pin or a set of pins that are e.g. dipped in a fixed reservoir containing fluid to be transferred and moving the pins into position to contact a usually soft substrate to form relatively large spots. Some of these instruments are known as "replicators". An example of a product produced by such prior pins would be a 22cm x 22cm bioassay plate carrying 0.6mm diameter spots

motion toward and away from the surface, the apparatus adapted, by repeated action, to deposit the drops of fluid precisely in a desired array, preferably the apparatus being computer controlled.

Preferred embodiments have one or more of the following features.

The drop-carrying surface has a diameter less than 375 micron, preferably less than 300 micron, preferably between about 15 or 50 micron and 250 micron.

The drop-carrying surface is bound by a sharp rim that defines the perimeter of the drop of fluid.

The deposit device is a pin or pin-like structure having an end surface that carries the fluid drop, preferably the pin or pin-like structure having sides that intersect with the end surface to define a sharp peripheral drop-defining rim.

Another broad aspect of the invention is an apparatus for depositing fluid drops on a receiving surface per se, comprising a deposit device and a fluid source which are cooperatively related to provide to a drop-carrying surface of the deposit device a precisely sized drop of fluid, the deposit device being a pin or pin-like structure having an end surface that serves as the drop-carrying surface, the pin or pin-like structure having sides that intersect with the end surface to define a sharp peripheral drop-defining rim.

Preferred pins or pin-like structures have an end surface that is generally flat and side surfaces that are cylindrical and smooth.

In preferred cases the deposit device is mounted for compliance in the direction of the deposition motion when the deposit device engages the receiving surface, preferably the deposit device being compliantly displaceable by overcoming resistance of a resilient member or weight, preferably, when the deposit device is

surface, comprising a deposit device and a fluid source which are cooperatively related to provide to the deposit device a drop of fluid, transport mechanism for positioning the deposit device over a receiving surface and drive mechanism for moving the deposit device, relatively, in deposition motion toward and away from the receiving surface, the apparatus adapted, by repeated action, to deposit the drops of fluid in a desired array, the fluid source being a mobile fluid storage device that is movable relative to the array of deposit locations, the fluid storage device being constructed and arranged to resupply the deposit device at various locations along the array.

In preferred embodiments employing a mobile storage device, the deposit device and the mobile storage device are constructed to supply drops to the deposit device in the immediate vicinity of the deposit locations for respective drops, preferably the mobile fluid storage device and the deposit device being coupled for transverse motion relative to the array and decoupled for movement of the deposit device toward and away from the receiving surface.

In many cases the mobile storage devices are preferably constructed and arranged to be replenished from a remotely located large reservoir.

In many cases a mobile storage device holds a volume of fluid having a free surface into which the deposit device is lowered and raised to obtain a fluid drop, preferably the mobile storage device being constructed to store a multiplicity of isolated fluid volumes in the wells of a multiwell plate, the apparatus constructed to obtain its fluid from a selected volume of the plate.

In other important cases a mobile storage device defines a generally annular fluid retention surface or

device to a replenishment volume in which the member is immersed to receive a supply of fluid.

In certain preferred embodiments the deposit device is a pin or pin-like structure e.g. having one or more of the features described above, the pin or pin-like structure being mounted within the confines of an annular fluid retention surface and arranged to move axially relative thereto.

Preferably, fluid retaining surfaces of the annular storage device have a hydrophilic surface, e.g. a surface roughness of at least 1000 microinch or a surface energy greater that about 2500 mN/m, preferably the surface comprising tungsten, and preferably, e.g. when cooperating with the annular member to pick up a supply of fluid the drop-carrying surface or tip of the deposit device has a surface of surface energy greater than about 2500 mN/m, preferably the surface comprising tungsten.

The apparatus of any of the aspects and preferred embodiments described preferably include a cleaning system and a control system adapted to control relative movement of the deposit device to a depositing relationship to the receiving surface and a cleaning relationship to the cleaning system.

Another broad aspect of the invention is an apparatus for depositing an array of dots on a receiving surface, comprising a deposit device in the form of a pin or pin-like structure having an end surface capable of precisely defining a small drop of fluid, a source of fluid for the deposit device, mechanism for moving the deposit device relatively over an array of spaced apart deposit locations of a receiving surface, mechanism for repeatedly moving the deposit device, relatively toward and away from the receiving surface to deposit respective drops of fluid at selected deposit locations, a cleaning system, and a control system adapted to control relative

device independently, or mechanism for moving each deposit device simultaneously, relatively, toward and away from the receiving surface to deposit respective drops at respective deposit locations on the receiving surface.

For simultaneous actuation, preferably two or more deposit devices are mounted on a common support, driven by a common driver to deposit respective fluid drops on the receiving surface. In cases in which each deposit device is associated with a respective storage ring, the storage rings are also mounted on a common support, driven by a common drive; preferably the spacing of the rings corresponds to the spacing of a multiwell storage plate into which the rings are immersed for resupply. In cases in which the deposit device is lowered directly into fluid and raised to obtain its drop, preferably the spacing of the deposit devices corresponds to the spacing of wells of a predetermined multiwell plate, the multiwell plate being a mobile fluid supply that is constructed to accompany the deposit device across the substrate. In the case of supply rings or direct dipping of the deposit devices, preferably in the spacing corresponds to well-to-well spacing of wells of a 96, 384, 864 or 1536 well plate, or a spacing of 9 mm or a submultiple of 9 mm.

The various apparatus described preferably have one or more of the following features.

The deposit device and its mounting limits the force of engagement of the deposit device upon the receiving surface to less than 1 gram, preferably less than 0.5 gram, preferably to about 0.3 gram.

The deposit device has a natural frequency greater than 10Hz, preferably greater than 20Hz.

The motion of the deposit device toward and away from the receiving surface is damped, preferably by

away from the receiving surfaces to deposit respective dots at respective deposit locations on the surface, preferably the fluid source being a mobile fluid storage device separate from the deposit device, which is generally movable over the array of deposit locations, the fluid storage device being constructed and arranged to resupply the deposit device at various locations with respect to the array.

In certain preferred embodiments of this aspect also, the deposit device is a slidable pin or pin-like structure constructed and arranged to dip into a volume of fluid carried by a mobile storage device, preferably the storage device being constructed to store a multiplicity of isolated fluid volumes, the apparatus constructed to move the supply device relative to the deposit device to select the fluid to be deposited, preferably the storage device being a 96 well plate or a plate having a multiple of 96 wells, and also preferably including at least one driven stage for moving a selected well of a mobile multiwell plate into registry with the deposit device under computer control for enabling motion of the deposit device to dip into and out of the preselected well to provide a drop of the selected fluid to the device.

In other preferred embodiments the mobile storage device is an annular ring as described above.

In embodiments in which the deposit device is a pin or pin-like structure, it is preferably positioned by engagement with a surface of revolution whose axis is disposed at a predetermined relationship to the receiving surface or substrate, preferably the surface of revolution being in the form of a supporting ledge that supports the device from moving in its assembly in the direction toward the receiving surface, but from which the device is free to lift in response to contact of the

described, mounted for motion together in response to a common actuator, preferably the deposit devices comprising deposit pins or pin-like structures.

Another broad aspect of the invention is an apparatus comprising a mobile fluid storage device separate from a deposit device and generally movable over an array of deposit locations, the fluid storage device being constructed and arranged to resupply the deposit device at various locations with respect to the array, in one case, preferably the mobile fluid storage device being constructed to store a multiplicity of isolated fluid volumes, the apparatus constructed to move the mobile storage device relative to the deposit device to select the fluid to be deposited, preferably the deposit device being a pin or pin-like structure constructed and arranged, under computer control, to dip into a selected volume of fluid carried by the mobile fluid storage device, preferably the mobile fluid storage device being a multiwell plate having 96 wells or multiples of 96 wells, or a spacing of 9 mm or a submultiple of 9 mm and preferably the apparatus including a driven stage for moving the fluid storage device into registry with the deposit device under computer control for enabling dipping of the deposit device into a preselected fluid volume; in another case preferably the mobile storage device is an annular ring that retains a supply of fluid by surface tension.

The invention also features the method of use of all the described apparatus in depositing fluid drops, especially the fluids mentioned in the specification.

Another broad aspect of the invention is a method of depositing a biological compound on a substrate or causing biological compounds to interact with another substance at a predetermined position on a substrate, including the step of depositing at least one of the

be in proximity to the deposit pin or pin-like structure, or a plate visited by an annular supply ring.

Another broad aspect of the invention is a method of producing arrays of fluid dots comprising providing an array of compliant deposit devices according to any of the foregoing claims, the devices preferably being in the form of pin or pin-like structures, the devices having spacings corresponding to the well spacing of a 96 well plate, or a plate having a multiple of 96 wells or a spacing of 9 mm or a submultiple of 9 mm, according to a sampling plan, preferably either dipping mobile annular supply rings into wells of the plate or dipping the devices directly into wells of the plate with which the device is registered to provide fluid drops on the devices and transferring the drops to respective locations in substantially denser arrays on a receiving surface, preferably the drops being deposited on a microscope slide in a pattern of square arrays.

In the various methods, preferably drops of fluid are deposited under computer control, by moving at least one compliantly mounted pin or pin-like structure having a drop-supporting surface of diameter less than about 375 microns, preferably less than 300 microns, preferably less than 250 micron, to a selected position and depositing, with the pin or pin-like structure, a desired material.

In the various methods, preferably the receiving surface is fragile, or soft, preferably the receiving surface is porous or microporous or fibrous, preferably comprising nitrocellulose, nylon cellulose acetate or polyvinylidine fluoride or a gel, preferably the member defining the soft or fragile receiving surface being mounted on a rigid carrier member, either directly or upon an intermediate soft or resilient buffer member.

Brief Description of the Drawings

In the Figures:

Fig. 1 is a free body diagram of a deposit pin that is laterally constrained by being loaded against a reference surface. Fig. 1 also illustrates alternate mobile sub-reservoirs with which the deposit pin is employed.

Fig. 1A is a cross-section taken on line 1A-1A of Fig. 1.

Figs. 1B and 1C are views similar to Fig. 1A illustrating other pin loading arrangements.

Fig. 1D is a diagrammatic perspective view of a deposit pin that has a conical surface mated with a complementary seating surface of a support plate, while Fig. 1E is a diagram of a longitudinal segment of the support seating surface and Fig. 1F is a diagrammatic view with vectors that analyze the reaction forces of the support seat in response to lateral biasing applied to the pin.

Fig. 1G is a representation of a spring-mounted and damped deposit pin.

Figs. 2, 2A, 2B and 3 are diagrammatic side views of combinations of deposit pins and cooperating supports that provide lateral constraint of the pin, Fig. 2' is a preferred alternative arrangement of a portion of Fig. 2, while Figs. 3A and 3B are cross-sectional views taken on respective section lines of Fig. 3.

Fig. 4H is a perspective view of a deposit pin mounted by a pair of parallel spring flexures by which it is laterally constrained and prevented from rotating about its own axis, the pin acting vertically through a mobile sub-reservoir while Figs. 4A and 4B are partial cross-sectional views of other deposit pin mounting constructions employing pairs of spring flexures.

Fig. 9I is a cross-sectional view of a presently preferred annular sub-reservoir device suitable for picking up low viscosity fluids from a narrow main supply as illustrated in Fig. 9H, while Fig. 9J is an end view of the device of Fig. 9I.

Fig. 9K is a magnified view of a pin and ring assembly in which the fluid contact surfaces are specially coated while Fig. 9L illustrates on a less magnified scale the immersion of the assembly for pickup of a local fluid supply and Fig. 9M, similar to Fig. 9K, illustrates the fluid load that is picked up by the assembly.

Fig. 11 is a perspective view of a multi-pin deposit head mounted for cooperation with a multiwell mobile supply reservoir while Fig. 11A shows the same elements as Fig. 11 in a supply relationship.

Fig. 12 is a diagram of an operable pin pattern of micro deposit pins while Fig. 13 illustrates the initial relationship of the pins to a standard 96 well supply plate.

Fig. 14 defines a useful sampling sequence for the pins of Fig. 12.

Fig. 15 illustrates a pattern of separated squares on a microscope slide which the pins of Fig. 12 can simultaneously address while Fig. 15B shows details of a square;

Figs. 16, 17, 18, 19 and 19B are views similar, respectively, to Figs. 12-15B, illustrating an arrangement employing a 12 pin pickup array used with a 96 well supply plate.

Fig. 20 is a perspective view of an assembled deposit pin constructed according to Fig. 2 combined with a respective supply ring; Fig. 20A is a similar view of a group of four such assemblies.

to which the pin is repeatedly exposed, the pin being sized and shaped to define and retain on its tip a drop of fluid from the reservoir, the drop containing only enough material to deposit a single dot.

The volume of the drop is typically determined by pin cross-section dimensions, shape and surface characteristics of the tip of the pin as well as by the viscosity and surface tension of the fluid to be deposited and the techniques by which the tip is supplied.

By providing the tip of the deposit pin with a sharply defined rim, it is found during repeated action that fluid drops of consistent volume are defined by the tip when all other factors remain constant.

Presently we prefer that the rim of the tip of the pin be "square", i.e. that, in profile, the end surface of the tip of the pin be substantially at a right angle to the side surface of the pin, and that the pin side surfaces be smooth. Preferably the pin is round in transverse cross-section, though it may be of other shapes. Under certain circumstances, as when depositing on porous substrates that readily receive the fluid, the end surface of the pin may also have a surface-tension enhancing surface to optimize the fluid acquisition capability of the pin. For example it may have a roughened surface, surface roughness of at least 1000 microinch, or be composed of high surface energy material, (surface energy greater than 2500 mN/m), such as tungsten.

It is found that arrays of fluid dots between about 20 microns to 375 microns can be deposited using biologic fluids of conventional concentrations, by employing deposit pins that have, in their tip regions, a wire or wire-like geometry of diameter (true diameter or cross-section dimension) between about .001 inch (25

fluid of generally corresponding dimension in the tightly packed arrays.

In the embodiment of Figs. 1 and 1A the deposit pin is carried on support 17. The pin, though laterally constrained at the time of engagement with the substrate, is mounted to be axially compliant, free to be displaced upwardly relative to the support when the pin encounters the substrate. When the support is lifted from the substrate the pin is free to return to its seat. The pressure applied to the substrate by the pin can be determined by the weight of the pin alone, or as supplemented by a spring or added weight. Preferably the pins are secured against rotation to ensure repeatability of position despite possible variations in shape of the pin due to manufacturing tolerances.

The details of preferred laterally referenced pins axially displaceable from their support are described later with reference also to Figs. 1B to 1F and to Figs. 2-2B, Fig. 2' and Figs. 3-3B. With the mountings shown, the movements occur accurately over a wide range of ambient conditions.

Another laterally referenced pin mounting arrangement is shown in Fig. 4, see also Figs. 4A and 4B. Pin 12 is supported by a pair of spaced apart, parallel, planar, cantilevered flexures 70, 72 that extend perpendicular to the direction of the desired compliant motion of the pin, to provide a parallelogram type of mounting that laterally constrains the pin to a reference position to enable landing of the pin at a precise location on the substrate. A soft landing occurs due to compliance provided by the flexures. To deposit precise arrays of dots as small as 25 micron (0.001 inch) diameter, an element of spring metal in at least one of the flexures 70, 72 ensures that the deposit pin returns to its original vertical position after each deposit.

of the pin support. The general principle of combined compliance and damping is illustrated in Fig. 1G. The actuator A acts through a highly compliant support spring Z, buffered by a damping device X, the moving assembly having a natural frequency in excess of 10 Hz. Where sliding action of weighted pins is employed, friction of the sliding surfaces can be employed to provide damping.

In embodiments that employ cylindrical deposit pins moving axially normal to the deposit surface, with spring mounting systems in which the weight of the pin is insignificant or counter balanced, it is observed that a spring support system for the pin with stiffness of less than 5 gram per millimeter deflection, measured at the pin, produces good results for cases in which the amount of pin deflection is a few tenths of one millimeter. In one particular case, a spring system having a spring deflection ratio of 3 gram/mm, deflected about 0.2 mm, resulted in deposition of dots of fluid of excellent, repeatable quality over a range of microscope slides.

Use of the deposit pins to deposit biological fluid or reagent on a rigid substrate is illustrated in the sequence of highly magnified, diagrammatic Figs. 5A-5E while Figs. 5F-5I illustrate use of the pins to deposit fluid dots upon a delicate, soft or porous membrane and the like.

In Fig. 5A, the deposit pin P is seen supporting a drop F of fluid obtained e.g. from a mobile sub-reservoir MW (Fig. 1) or sub-reservoir ring 14, (Fig. 1, 4). The pin moves under control of driver D toward a selected target point S on receiving surface R. In the case of dipping into sub-reservoir MW, surface tension effects hold fluid drop F in substantially semi-spherical form on the sharp-rimmed tip of the pin, see Fig. 7. When the tip is plunged through fluid held in sub-reservoir ring 14, the drop F is normally shallower, less rounded, see

wettability of the receiving surface and surface tension characteristics of the fluid. When surface R is hydrophobic, the deposited fluid drop may contract as it dries, while with wettable, fibrous, or porous surfaces, it may expand; in either event, the size of the deposited dot is determined principally by the size of the tip of pin 12.

In Fig. 5E the pin P, substantially devoid of fluid, moves away from receiving surface R, with a component of lateral movement, M. It is rapidly resupplied and proceeds to the next target point. The pin P is resupplied in important cases from a mobile local sub-reservoir that accompanies the pin across the substrate, e.g. the movable reservoir MW of Fig. 1 or the annular supply ring 14 of Fig. 1 or Fig. 4. The deposit cycle is then repeated at another lateral (X,Y) position to which the pin is moved.

Instead of placing deposits on a rigid, smooth substrate, the substrate may be a porous or microporous membrane or a delicate film such as nitrocellulose, cellulose acetate, polyvinylidine fluoride (PVDF) or nylon, or it may be an agar gel or other gel. The particular substrate can be selected in accordance with the nucleic acid, protein or other transfer procedure being employed, and can be the same as or take into consideration the substrates previously used in development of historical reference data with which the results of the present experiment are to be compared. The compliance of the pin protects such fragile substrates from damage.

Action of a deposit pin in depositing biological fluid or reagent on a delicate, soft or porous substrate is illustrated in highly magnified Figs. 5F-5I. A delicate, relatively soft membrane D_M is supported directly on a rigid support R_S.

thin layer of microporous nitrocellulose film formed by casting, such as is available from Grace Bio-Labs Inc. under the trademark Oncyte® Film Slides. The process just described may be employed to deposit microarrays of fluids on such microporous film. Although the deposit-receiving surface is not rigid itself, by virtue of a rigid backing, the delicate membrane can be automatically processed, scanned, etc. by available robotic equipment that engage the rigid frame.

High density arrays of individual deposited dots as depicted in Figs. 5A-5D and 5F-5I are achieved by repeated deposit action of one or more deposit pins, with selected fluids being deposited at selected precise locations under computer control as further described below.

Figs. 5K-5N depict diagrammatically, in magnified scale, the depositing of one deposit upon another in a precisely aligned manner, made possible by the positional accuracy of the systems being described. Fig. 5K shows a deposit 100, as produced by techniques previously described employing a deposit pin, or by some other technique that achieves a known position. Fig. 5L shows deposit pin 12 having been indexed into precise alignment with dot 100, and lowered to engage fluid drop C with it. Fig. 5M shows the deposited second drop 104 still in fluid state while Fig. 5N shows dried second dot 106 deposited upon dot 100.

In similar fashion Figs. 5O-5R illustrate deposit of a relatively large spot of fluid using large deposit pin 120 and subsequent deposit of small drops using a smaller deposit pin 12 having a microtip. The large drop on the pin 120, in Fig. 5O, forms a large deposited drop 110, Fig. 5P, which dries to form a large dried spot 112, Fig. 5Q. Subsequently, small drops 114 are deposited in selected precise locations upon the large spot 112, Fig.

Large body 12a provides a downwardly-directed annular surface 12e that is engaged upon a support, to receive support force F_s that bears the pin's weight.

The assembly is constructed to enable a lateral bias force F_b to urge pin 12 against a pair of reference surfaces Ref_1 and Ref_2 which lie at an angle to each other as viewed in horizontal cross-section, Fig. 1A. These reference surfaces are arranged to resist movement of the pin in X and Y coordinates by applying reaction forces that have X and Y components. The combined X components of the forces provide resistance force F_r that balances bias force F_b. The reference surfaces are constructed to leave the pin free to move axially along axis A (Z direction), as by sliding, to provide axial compliance to the tip 12d when the substrate is encountered.

In certain practical embodiments, for mounting the pins, two vertically spaced horizontal plates 9 and 11, shown in dashed lines, are joined to form carrier 17 that moves in X,Y and Z directions for carrying the pin through deposit, cleaning and resupply motions. The upper plate 9 is at a selected distance from the lower plate and applies a constraining force F_c to constrain the angle of the pin, and hence the position of its tip 12d, within selected tolerance.

Lowering carrier 17 causes the precisely positioned tip 12d to engage substrate R, whether the substrate be found at level 20, 20a or 20b in the design range. Upon engagement with the substrate, the pin stops. Further downward movement of the carrier 17 occurs with the compliant pin remaining stationary above its seat, while reverse movement of the carrier causes the pin to reseat on its support. In this way the vertical movement of the carrier need not be controlled with high accuracy, and proper contact with the substrate

surfaces of revolution that define seating surfaces. Preferential seating upon a given segment of the seating surfaces may be achieved by the loading techniques previously described.

Figs. 2, 2A and 2B, show embodiments that employ a surface of revolution for supporting the pin in a laterally constrained manner.

Fluid deposit pin 12, (associated with a fluid supply such as mobile multiwell plate MW or associated supply ring 14), is constrained between upper and lower plates 9 and 11 of carrier 17. Carrier 17 moves in the direction of arrow Z for supply and deposit actions. In each case of Figs. 2, 2A and 2B, an enlarged portion of the pin 12a rests normally in a seat 13, 13a or 13b in plate 11 which bears the weight of the pin. The pin is free to be displaced relatively upwardly from its seat, as shown, upon contact of tip 12d with substrate 20. When the pin 12 rests in its seat, the X,Y position of tip 12d of the pin is defined by the degree of perfection of the pin 12, the relative distance to the upper supporting plate 9, the clearance between upper portion 12c of the guide pin and the guide hole 19 in plate 9, and, as indicated above, a preferred feature in the system that applies a definite (though permissibly slight) lateral bias of the pin to a given side of the engaging structure. For this purpose, in Fig. 2, compression spring 22 is disposed between upper plate 9 and upwardly directed ledge 24 of pin 12. The spring is fixed in position and applies its downward force with slight and predictable asymmetry relative to center axis A, to bias the pin to a given side, to ensure repeatable positioning of the pin on the same region of its seat on plate 11. Spring 22 is sized and arranged to also provide compliant pressure of tip 12d of the pin on the

of the pin within desired tolerances for precise deposit of dense arrays on the substrate 20.

It is seen from Figs. 2, 2A and 2B that the driven pin carrier structure 17 travels downwardly sufficiently to ensure that tip 12d can reach the lowest level 20b of the range of permissible levels. As in previous embodiments, the tip 12d is axially compliant in the sense that the pin can yield in position so that, when encountering the substrate, it exerts only a controlled light pressure on the substrate before it lifts from its seat.

In Fig. 3, the enlarged part 12a' of pin 12' and seat 13c have complementary conical surfaces normally engaged unless the tip 12d' is engaged with the substrate (but shown disengaged for purposes of illustration). The upper end surface 12g of the enlarged body portion 12a' is sloped in a selected direction as explained further below and a rigid ball bearing 38 bears on the sloped surface at a point offset from central axis A. A weight 39 rests upon ball 38, being housed by a bore in spacer block 41 upon which the upper and lower plates 9 and 11 are affixed. The spacer block is advantageously of a low-friction engineering plastic such as Delryn. The weight 39 is of selected size to adjust compliance to the degree desired for the deposit tip 12d' and to apply a slight turning moment M_o to the pin toward a portion of the conical seat, (see Fig. 1), via the eccentrically located ball.

As seen in the cross-section of Fig. 3A, weight 39 is of cylindrical configuration, free to rotate about axis A with turning of the ball to avoid applying undue drag. The main body 12a' of the pin, however, is of square cross-section and is disposed in square channel 42 in spacer block 41 of like configuration. This prevents pin rotation so that orientation of the upper end face

resin, e.g. Kapton® from duPont. An energy absorbent bonding agent, e.g., of thickness t of .002" provides a damping layer 77 between these resin sheets. The bonding agent may be a thin coat of rubber cement such as available from 3M as ID # 62-60065-4826-1 or 3M double sided tape # 927.

In an alternate construction shown in Fig. 4B, flexures 70b, 72b are identical, each being a sandwich of one metal layer 73 and one resin layer 75 bonded together by rubber damping layer 77. Compliance similar to that of Fig. 4A is achievable with the selection of material of appropriate thickness, such as either a stainless steel layer 0.0016 inch thick or a copper-beryllium layer 0.0022 inch thick, bonded by the damping layer to a polyamide layer 0.005 inch thick.

The physical properties of the flexures can also be tailored to the particular need by change in geometry of the flexures. An example is the provision of cutouts.

In manufacture, a large-area bonded sandwich of all three materials may be fabricated and the shape of the flexures can then be produced by photo etching the desired outline and any cutouts.

In a cluster of deposit pin assemblies of the type shown in Figs. 4, 4A and 4B, such as shown in Figs. 24A and B described below, with 9mm spacing of pins to correspond with the spacing of wells in a 96 well plate, the flexure elements are preferably 8mm wide and 22mm in length, and two or more of the pin and flexure assemblies are mounted in parallel, side by side, at 9mm pin spacings. Preferably two sets of such assemblies, disposed head-to-head as described below, are employed at 9mm pin-to-pin spacings, so that an X,Y array of pins is achieved.

B. Mobile Fluid Reservoirs

receiving surfaces. Data that correlates locations with respective specimens is recorded in memory and used in subsequent scanning or reading.

The technique of using a deposit tool that accurately sizes each individual drop, such as the deposit pin with square rim profile at its microtip, combined with a mobile local sub-reservoir that accompanies the tool and carries a volume sufficient to supply a sequence of deposits, has a number of important advantages. The technique, based on small motions, saves time in avoiding repeated travel to a central supply; it avoids evaporation losses of long travel, so that the drop created can be very small and the deposited array very dense; and the dots can be kept consistent in size and concentration or biological content across the array of dots being deposited. The time overhead involved in cleaning, transporting and picking up the specimen is kept small so that, overall, deposits can be made very fast, inexpensively and of desired small size.

In this way a large number (for instance ten to one hundred) identical microscope slides or membranes can readily be prepared by repeated motions over an array of the slides or membranes. Each substrate can carry dots of many different fluids based upon resupply of the sub-reservoir from different wells of a number of multiple well plates introduced to the system.

The sub-reservoir and the deposition device are decoupled, in being movable relatively to one another for resupply and for deposit, as well as being coupled or at least coordinated, to move laterally over the receiving surface to produce the series of deposits. The sub-reservoir can move into a resupply position, e.g. by immersion into a well, or under a suitable pipette. It can be made to hold sufficient fluid in excess of that required for the sequence of deposits, or to expose a

position, the end 12d of pin 12 is drawn above the lower surface of the retained fluid R_f held by surface tension effects between the internal surfaces of ring 14. This is shown in Fig. 9A. (The pin, for illustration, is shown withdrawn fully above the retained fluid R_f, although that is not necessary.)

Comparing Fig. 9A with Fig. 9, by downward movement of the pin tip from above the lower surface of the retained fluid R_f (Fig. 9A), to below that surface (Fig. 9), the tip of the pin, with its sharply defined rim, picks up from the retained fluid R_f a precisely sized volume of fluid as drop F. The drop is then deposited in the sequence shown in Figs. 9C and 9D.

At the resupply position of Fig. 9E, the annular ring 14 is moved downwardly by its support rod 15 for immersion in the well of the supply plate while the pin 12 remains stationary at a higher elevation, Fig. 9E, or it may assist in the resupply action, see Fig. 9L, described below. The ring is moved by associated driver D_1, Fig. 9A.

At cleaning and drying stations, Figs. 9F and 9G, the lower surfaces of the pin 12 and ring 14' are shown vertically aligned (the ring here shown as a cylindrical ring).

At washing station, Fig. 9F, the ring and pin may both be subjected to reciprocation in the vat of cleaning solution in the same or opposite vertical directions to assist the cleaning process. The wash station may be an ultrasonic bath.

The multipurpose station illustrated in Fig. 9G is sized to receive deposit pin 12 and supply ring 14. It has an annular nozzle 200 directed inwardly against the pin and ring to subject the parts to a conical flow from fluid sources such as compressed air, pressurized liquid and aerosols. The flow is directed past the parts 12, 14

One example is the provision of internal surface roughness of at least 1000 micro inch. This causes the central region of the ring effectively to have superior hydrophilic properties, i.e. a better "grip" on the fluid by surface tension effects. This permits the uplift of a suitable volume of fluid from a container of approximately mating shape. The exterior surface of the ring may also be provided with a fluid retentive surface to supplement surface-tension effects of the ring, to compete with the retentive properties of the well.

Surface roughness of the internal or exterior surface can be obtained by sanding, broaching or by machining the part on a lathe with a tool or a tap. The ring can also be manufactured from suitably coarse particulate material that is sintered or molded with a binder. Likewise a durable coating can be applied such as formed by carbide particles.

As shown in Fig. 9I, in one preferred embodiment, a cylindrical ring 14A of stainless steel has a height h of .050 inch, an inner diameter of .060 inches and an outer diameter D of 0.080 inch. It is tapped by a tool having 80 threads per inch, that produces a thread height d and pitch p of 0.012 inch, (the internal diameter is much larger than the diameter of the deposit pin P with which it is used). As shown in Fig. 9H, an annular ring provided with surface roughness in this way is effective to pick up liquid from the conical well of a PCR plate. Despite the desired surface tension effects produced by the internal ring surface, it has smooth surface increments that promote good cleaning.

Also shown in Figs. 9I and 9J is support rod 15 e.g., of 0.15 inch diameter stainless steel wire, soldered or spot welded at 104a to the exterior of the ring. It drives the ring in its motions, and provides

forms the cross-sectional shape of the ring is selected in relation to the properties of the fluid (e.g. viscosity and surface tension), the number of deposits to be made from a given fluid charge in the reservoir ring, and the size of the deposit pin that is to move through the ring.

The size and shape of the deposit pins that cooperate with these and other sub-reservoirs also vary depending upon the application. It is possible to employ pins of various transverse cross-section, e.g. square or hexagonal or even rectangular or oval cross-section of equivalent area to round cross-section pins. Especially for small dots, the pins may advantageously have stepped transverse cross-sections, e.g. may have an extremely small cross-section at the deposit end, to size the deposited drop, stepped to a larger cross-section in the main body, for providing structural stability. An example is shown in Fig. 2B.

For implementing the broad concept of a local, mobile supply, other techniques than those shown can be employed. An example is a large dip rod, an enlarged version of a deposit pin, from which a large drop depends, which travels with the pin and is visited by the pin by a suitable motion, such as rotation.

C. Operating Systems

Some advantageous, novel operating systems that implement the foregoing principles will now be described.

DIP & DOT SYSTEM

The mobile reservoir MW shown in Fig. 1 is shown multi-celled, to represent a multi-well plate. Under computer control, an appropriate X,Y stage brings the chosen fluid resupply well in alignment under the pin. The pin is then controlled to descend, make contact with

Rail support 60 of Fig. 11, constructed e.g. to support linear motor movement in the Y coordinate is mounted on an X stage 62, motor not shown. As shown, Y direction linear motors #1 and #2 respectively drive the pin assembly P_A and the multiwell reservoir MW in the Y direction. The reservoir has a secondary linear motor X_2 driven by a further driver for X movement of the reservoir relative to the pin assembly. The pin assembly also has Z freedom of controlled movement, driven by a further driver Z.

Under computer control, the multiwell reservoir separates in the Y direction from the pin assembly as shown in Fig. 11, and the Z stage is actuated to cause the pins to form deposits upon substrate R. Then, Fig. 11A, the multiwell reservoir moves under the raised pins into appropriate alignment, employing both Y_2 and X_2 motions under computer control. By Z motion the pins P_A dip into the commanded wells for resupply. The pins rise again, the multiwell reservoir moves laterally with Y_2 motion out of the way and the deposit process is repeated at new targeted X,Y location of the pins on substrate R or R_1. While this mobile reservoir technique is useful with pins of any construction, the advantage of high accuracy of the linear motor indexing system is enjoyed when the pins are constrained in space to a highly accurate repeatable position relative to their carrier, either with the high density pin arrangements made possible by the structures described with respect to the various Figs. 1, 2, and 3 or the flexure mountings that have been described with respect to the Figs. 4.

MULTIPLE PIN PATTERNS

In the preferred embodiment of Fig. 12, two rows of 4 pins P, preferably constructed according to Figs. 1-4 are spaced apart in a 9 mm square grid pattern matching

plates, e.g. arrays of 20 micron to 375 micron diameter dots with similar spacing between dots, using all fluids in the plate.

Just as the pins are located on 9mm centers, the square arrays themselves are distributed on 9mm centers over the face of the substrate. By following the pickup sequence shown in Fig. 14 (rows 1 through 2, and columns A through H), by repeated samplings, all wells are visited, the pins being conveyed under computer control to the cleaning station, not shown, between change of fluids. The contents of the multiwell plate or a number of plates are thus distributed from the low density distribution of wells in multiwell plates to high density arrays.

Similarly, referring to Figs. 16-19, again using 9mm pin spacing, with two rows of 6 pins each, a sequence of samplings from the wells under computer control collects samples from all wells and uniquely distributes them as high density array deposits in 12 squares on the microscope slides or other substrate with array and slide dimensions as shown in Fig. 19.

The benefit of such groups of pins is to create a large number of deposited dots simultaneously on one or many microscope slides or substrates. This can substantially reduce the time and cost required to create high density arrays.

The assemblage of pins on a 9 mm square grid can also be used to transport fluid from plates with well spacing constructed on a square grid that is based on sub multiples of 9mm, such as plates with 384 wells or 864 wells or 1536 wells, etc. The high accuracy of the computer controlled gantry system enables accurate placement of the selected wells with respect to the pins, and the pins with respect to the receiving substrate.

rails for X and Y motion provide a complete array-forming mechanism. Fig. 22 illustrates a commercial realization of the design which attaches to the Y stage linear motor of Fig. 21.

Referring to Figs. 23, 23A, and 23B, deposit pin 12 in this case is mounted on a parallelogram, cantilever construction. Spaced-apart planar flexures 60 are mounted in parallel on a mounting block 62, sandwiched by mounting plates 64 and 67 against the intervening block 62. These flexures extend in cantilever fashion to intermediate block 66, arranged to be engaged by pusher rod 68 associated with a prime mover 76, Fig. 23B. Extending further in cantilever fashion from intermediate block 66 are parallel flexures 70 and 72 which include cut-outs 74 that render the flexures weak and highly flexible (compliant). At the end 79 of weak flexures 70 and 72 is mounted deposit pin 12. The condition of no force being applied to the structure is shown in Fig. 23 in which the flexures are horizontal, the weight of the pin being borne by the mounting structure. In Fig 23A, force applied in the direction of the arrow 68 results in deflection of the stiff flexures 60 to the shape shown, such that block 66 remains parallel to the receiving surface and deposit pin 12 remains in perpendicular position to the receiving surface. (Thus the relatively stiff flexures 60 and the associated driver perform the function of a precision stage.)

The flexures may be comprised of synthetic resin cut to shape, e.g. polyamide resin, available as Kevlar™, from duPont, or etched from thin spring metal such as beryllium copper or stainless steel. Advantageously both the stiff and weak flexures are formed continuously from a single sheet of spring stock.

The cluster 28 may step to a selected X or selected X,Y position, at which a number of different motions under computer control may be caused to occur, picking up and depositing fluid in any order at any location desired. Such a cluster constitutes a particularly versatile tool when employed with conventional microtitre plates.

In such embodiments the aliquot carrier rings 14 and pins 12 are spaced in the cluster at 9mm center-to-center distances or multiples thereof to facilitate operation with 96 well plates (in which the wells are spaced at 9 mm on center intervals, with 8 rows of 12 holes). Higher density plates also employ this configuration and have the same footprint but employ more holes, 16 x 24, with hole-to-hole resistance of 9/2 mm, to provide "384 plates". The arrangement of Fig. 24 enables use of the higher density plates with existing automated 96 well plate handling equipment. The system described can be employed with both types of plates, as well as any arbitrary arrangement.

The versatility of the cluster of independently operable deposit pins is illustrated by the following examples.

Sub-reservoir rings, e.g. set at 9mm center-to-spacing, may be indexed in X,Y direction along with their pins and the rings may be driven down (or dropped) simultaneously for supply or resupply from four wells of a conventional 96 or 384 well plate, in an action similar to the systems previously described.

After suitable indexing, the four pins may be driven down simultaneously to form deposits at four places, in the same format as the supply plate.

Alternatively, during resupply, one sub-reservoir ring may be dropped to pick up material from a selected well while all others remain in their passive positions.

ARRAYER

The gantry of an arrayer, now to be described, can carry one deposit head, a cluster of independently operable single pin heads, or a multiple pin head of the various designs described above. Combinations of these are also possible.

Fig. 25 is a perspective view of a slide preparation machine for preparing microscope slides or other substrates such as delicate soft or porous membranes carried on rigid supports. Its function is to rapidly deposit a high density array of fluid dots of different compositions on a number of identical substrates, employing the microdot technology of the present invention. As shown in Fig. 25, there are four 96 well supply plates 31, serving as the central fluid source for resupply of mobile fluid storage devices.

Horizontal base plate 200 provides a support structure to hold the operating components. Fastened to base plate 200 are vertical sub plates 210, 220, 230 and 240. Fastened to these plates is a dual axis motion system 250, comprising X and Y axis devices 260, 270 for providing X and Y motions, in a parallel plane.

The guide rails of the X and Y axis devices, 260, 270 are parallel to base plate 200, to carry deposit cluster 28 in X,Y motions in a plane parallel to base plate 200.

The X axis device 260 is a commercial device available from Adept of Japan. It moves at a high rate of speed in a controlled manner using a rotary servo motor with a drive screw and a shaft position encoder, employing digital and analog technology. Carried by X-axis device 260 is an orthogonally arrayed Y-axis device 270 which is a smaller version that operates in the same manner as the X-axis device.

For deposit on microscope slides including slide-like rigid members carrying delicate, soft membranes, the slides are fastened to the table, or placed in register with guides in a known position. Features on the base plate of the machine locate the slides in predetermined orientation.

In the preferred embodiment of Fig. 25A, microscope slide MS rests upon slide support 30, having one end engaged with stop 31 and its other end engaged by a spring wire 32. Wire 32 extends from a support screw on the bottom side of support 30, through a hole 33 in support 30, and is biased to the right in the figure to engage the slide MS to urge it against stop 31. The spring pressure is sufficient to hold the slide MS endwise in secure, accurate position despite vibrations that occur during operation of the machine.

The slides are mounted side-by-side in subgroups of seven slides, with their thin long edges engaged with one another. The seventh slide's position is dependent only upon the tolerances of the preceding six slides. By having such sub groups, one is assured that the array is properly located. The computer is enabled to "talk" to the slide and to record information, as in bar code. The bar code reader is mounted on the servo drive 270 of the Y axis and adjacent to the deposition means 28. The sequence starts with filling the multiplicity of rings of the deposition device, and is carried out according to the control procedure of Fig. 27.

For use in high volume production contexts, the system described in the foregoing Figures 1-27 preferably employs a rapidly moving, laterally constrained, axially compliant pin, in a deposit cycle of less than 0.1 second, in which impact and vibration is minimized, with the natural frequency of the system more than 10Hz, in

deposited in registry with previously deposited spots or microdots of biological material, and vice versa.

Among the many biological materials that may be spotted at high speed are fragments of nucleic acids, e.g. DNA, RNA or hybrids such as PNA (peptide nucleic acid), PCR (polymerase chain reaction) products, cloned DNA, and isolated genomic RNA or DNA, as well as synthetic analogs.

Also included are restriction enzyme fragments, full or partial length cDNA, mRNA or similar variations thereof, proteins such as protein receptors, enzymes, antibodies, peptides and protein digests; carbohydrates; pharmaceuticals; microbes including bacteria, virus, yeast, fungi, and PPLO; cells and tissue fragments; lipids, lipoproteins, and the like; plastic resin polymers, small particulate solids in suspension, etc.

The deposition system may also be employed to deposit catalysts, reagents and encapsulents upon previously deposited material of any of the types above or, as mentioned below, to create an array of sites or micro-wells for later reaction or growth of such material, or to assist in neutralizing or cleaning the deposit or reaction sites, as in the case of highly toxic or virulent substances.

The most basic use of the arrayer is to create high density arrays of nucleic acid on a porous or solid, flat surface, generally a microscope slide or slide-like support. Deposit on fragile or soft surfaces such as microporous membranes or gels, glass cover slips, plastic surfaces, and wells of a microplate, or any substrate, which may be previously coated or derivatized, may serve as a recipient surface.

In particular, membranes and gels are desirable to enable high density analysis with automatic equipment, using materials familiar to the field, on which much of

well, statistically, can deposit the desired quantity, which then can interact with nutrient, experimental drug, etc. in the well.

The concept of insertion is extended to include the deposit of particulates in suspension, for example, to deposit cells and then afterwards, deposit a suspension of particles of asbestos or precipitated silica or other solids of interest, to investigate effects of the particles upon the cells. These are examples of inexpensive, highly accurate, micro-controlled experiments that can be conducted at efficient speeds using the dedicated aliquot reservoir and deposit pin.

In many important cases the fluid or liquid carrier of the deposited spot evaporates and the biological or other material carried in the fluid stays in place by adhesive or bonding properties of the dried material. In other cases, the spotting technique is useful to deposit fluid that remains in a fluid state, for instance, as mentioned, to deposit cells into wells with fluid nutrient medium that enables the cells to continue to live.

In many cases it is important to know where a deposit is and that it will stay in the deposited position when covered by a common reagent. Steps can be taken to secure the deposit in position, for instance, with DNA, by exposing the deposit to UV radiation to crosslink the material or to use a derivatized surface that produces crosslinking between e.g. DNA and the surface on which it is deposited. An example is a silenated surface coated with E.S. aminosilene, to provide a positively charged surface which binds, by ionic or electrostatic forces, with negatively charged deposits such as DNA.

a relatively small exposed surface-to-mass ratio, which limits evaporation. Transport from that volume of the tiny sample on the tip of the pin, over a short local distance, limits exposure of the tiny sample to evaporating conditions until the dot of fluid is deposited.

Where desired, the operating deposit mechanism is shielded from windage by a protective shield mounted on the head to move in X-Y directions with the deposit mechanism, to further limit evaporative loss. In another case, the environment in which the system operates is controlled, e.g. at high humidity, or high partial pressure of the volatile substance, to limit evaporative loss, or at particular controlled conditions, e.g. controlled temperature and humidity, to favor the deposition process or the operation of the system itself.

Time-based sampling to evaluate chemical reactions or growth stages can be performed automatically without attendance of laboratory personnel. In one example, the fluid carrier ring through which the pin operates is employed as a reaction vessel from which samples of the continuing reaction are periodically taken by an associated deposit pin, and deposited for later inspection.

In this or other examples, at prescribed time intervals, another pin moves through its ring to deposit an inhibiting reagent to halt the reaction or growth that is occurring at a respective location on a substrate. By doing this at timed intervals over different locations on an array of identical reactions, a fixed array that represents the sequence of conditions at the various time intervals is preserved for later examination.

In another method employing the deposit system, an etchant fluid is provided in a local reservoir ring. The

wide field scanning microscope such as available from applicant. The principles described here enable wide area arrays to be formed of very high density over the mentioned wide range of fluids and conditions, while wide area scanning microscopes enable commensurate accurate and inexpensive reading of the results achieved with such wide arrays. The wide area and precision capabilities of each system and method, in combination, complements the other to achieve an enabling, significant advance in microdot reaction and analysis. Use of an array of flurophor-tagged components, such as is employed in biotechnology, or flurophor-tagged contaminants, followed by reading with a suitable wide field scanning microscope that excites the flurophors is of particular advantage.

F. Useful Additional Features

In one embodiment, an inductive heater station is provided to which the deposit mechanism can travel under computer control. In this case the substance of the reservoir ring and the deposit pin, or at least the surface portions of these devices, are comprised of electrically conductive material capable of having electrical currents induced by an alternating field of the induction heater. Under computer control, the reservoir ring and the pin are delivered for a momentary pause in the heater, for heating based on resistive (I^2R) losses by the induced electrical currents, for instance to sterilize the reservoir ring and deposit pin or to stop bioactivity in the fluid material retained on the instruments.

In another instance, a reservoir ring containing a charge of reactant fluid, which is desired to be heated, can be introduced to the inductive heater, and the fluid is heated by heat-transfer to the fluid from the inductively heated ring. Such heating can be employed to

capabilities. Under computer control, an X-Y carriage of the system is moved to select a desired head which is carried across the substrate to perform its function. In some instances the device selected may be a sub-reservoir ring from a set of such rings that have different internal diameter or are formed of different wire or ribbon sizes, or are of different sizes to enter different wells, etc. These provide a variety of carrying capacities for fluids of different viscosities or for use with deposit pins of different sizes. Likewise, different sizes of deposit pins can be selected from a set of pins to vary the size of the spot to be deposited. Heads can also be selected that provide other devices for preparing for or conducting experiments or for the production of reference or diagnostic well plates and slides.

In some cases the selection and use of devices can be conducted under complete computer control to enable automatic performance of a multi-task experiment un-attended by the technician.

In addition to depositing spots of fluid upon a standard microscope slide, and upon porous or soft membranes and other delicate substrates, it is possible and advantageous to deposit spots on substrates of significantly larger area and on other substances and on surfaces having special formations, for instance upon substrates having micro-cavities that have been formed by the instrument itself, by one of the techniques described above. Plates delivered with the micro-cavities preformed in the substrate may also be used, and aligned for deposit of fluid by automatic controls of the instrument, or the control system of the unit is advantageously provided with a vision system that "reads" the location and pattern of the array of micro-wells, and

1. An apparatus for depositing fluid dots on a receiving surface in an array suitable for microscopic analysis reaction, or the like, comprising a deposit device and a fluid source which are cooperatively related to enable the deposit device to precisely size a drop of fluid of small diameter on a drop-carrying surface of the device, transport mechanism for positioning the device at a precisely referenced lateral position over the receiving surface and drive mechanism for moving the deposit device, relatively, in deposition motion toward and away from the surface, the apparatus adapted, by repeated action, to deposit the drops of fluid precisely in a desired array, preferably the apparatus being computer controlled.

2. The apparatus of claim 1 in which the drop-carrying surface has a diameter less than 375 micron, preferably less than 300 micron, preferably between about 15 and 250 micron.

3. The apparatus of claim 1 or 2 in which the drop-carrying surface is bound by a sharp rim that defines the perimeter of the drop of fluid, preferably the surface defined by the end of a pin or a pin-like structure which has sides that intersect with an end surface to define the rim, preferably the end surface of the pin or pin-like structure being generally flat and the side surfaces are cylindrical and smooth.

4. The apparatus of any of the foregoing claims in which the deposit device is mounted for compliance in the direction of the deposition motion when the deposit device engages the receiving surface, preferably the deposit device being compliantly displaceable by overcoming resistance of a resilient member or weight,

arranged to resupply the deposit device at various locations along the array, preferably the deposit device and the mobile storage device being constructed to supply drops to the deposit device in the immediate vicinity of the deposit locations for respective drops and preferably being coupled for transverse motion relative to the array and decoupled for movement of the deposit device toward and away from the receiving surface.

7. The apparatus of claim 6 in which a mobile storage device holds a volume of fluid having a free surface into which the deposit device is lowered and raised to obtain a fluid drop, preferably the mobile storage device being constructed to store a multiplicity of isolated fluid volumes in the wells of a multiwell plate, the apparatus constructed to obtain its fluid drop from a selected volume of the plate.

8. The apparatus of claim 6 in which a mobile storage device defines a generally annular fluid retention surface, and the deposit device is constructed to move within the annular retention surface from retracted to extended positions, in the retracted position the drop-carrying surface of the deposit device being retracted from the surface of fluid retained by the annular surface of the storage device, and in the extended position the drop-carrying surface of the deposit device being projected through and beyond the surface of the retained fluid, preferably the deposit device being a pin or pin-like structure mounted within the confines of the annular surface and arranged to move axially relative thereto, and preferably the member defining the annular surface being associated with a driver that moves the member relative to the deposit device to a replenishment volume in which the member is

surface tension effects, the nozzle directed to dislodge retained fluid, to clean or to dry the respective parts.

10. The apparatus of any of the foregoing claims, comprising a set of at least two of said deposit devices, at least one fluid source for providing a drop of fluid on each deposit device, and mechanism for moving the deposit devices together transversely over an array of spaced apart deposit locations of the receiving surface, preferably there being at least four of the deposit devices comprising a deposit head, preferably including mechanism for moving each deposit device independently, or mechanism for moving the deposit devices simultaneously, relatively, toward and away from the receiving surface to deposit respective drops at respective deposit locations on the receiving surface.

11. The apparatus of claim 10 in which two or more deposit devices are mounted on a common support that is driven by a common driver to deposit respective fluid drops on the receiving surface; preferably each deposit device being associated with a respective annular storage ring, the storage rings being mounted on a common support, driven by a common drive, preferably the spacing of the rings corresponding to the spacing of a multiwell storage plate into which the rings are immersed for resupply; or preferably the spacing of the deposit devices corresponds to the spacing of wells of a multiwell plate fluid source from which the deposit devices obtain fluid directly, preferably the multiwell plate being a mobile fluid supply constructed to accompany the deposit device across the substrate; preferably in either instance, the spacing of the wells of the multiwell plate corresponding to well-to-well

preferably the surface of revolution having a surface of form substantially matching the form of the portion of the device disposed to engage it, preferably the surfaces being respectively conical, each preferably conforming to a portion of the surface of a right cone.

15. The apparatus of any of the preceding claims in which the deposit device is urged against a reference surface, in which the assembly applies a lateral force or turning moment to the deposit device, preferably the force or the turning moment being applied by a spring that bears eccentrically on the device or by a pushing member engaged with a remote end of the deposit device, one of the engaged end and pushing member comprising a surface set at an acute angle to an axis of the device, and the other of the surfaces comprising a convexly curved surface engaged upon the angled surface, preferably the convexly curved surface defined by a confined ball that bears against the inclined surface, preferably by being pushed by a weight.

16. The deposit apparatus of any of the preceding claims in which the deposit device is in the form of a pin or pin-like structure, wherein a structure prevents rotation of the deposit device about its own axis; preferably the pin or pin-like structure being confined in a complementary space that prevents its rotation about its own axis; or preferably a detent prevents rotation of the deposit device, preferably the detent comprising part of a coil spring which surrounds and is frictionally secured to the pin or pin-like structure, a protrusion of the spring engaging a stop surface that prevents the rotation, preferably the spring also providing axial compliance to the pin or pin-like structure.

dipping the pin or pin-like structure in fluid, or the deposit device is supported above the substrate at the deposit location within an annular ring holding fluid by surface tension, and the pin is moved through the ring in the manner that a relatively small drop of the fluid supply is held by to the end of the pin or pin-like structure by surface tension, preferably the pin providing a drop-carrying surface bound by a sharp rim that sizes the drop.

20. The method of claim 18 or 19 in which the fluid to be deposited is fluid selected from a group of fluids disposed in a multiwell plate, in cases in which an annular ring supplies the deposit device, the ring traveling to the multiwell plate for resupply, in cases in which the deposit device dips directly into wells, preferably the multiwell plate being mobile, mounted to move across the substrate to be in proximity to the points of deposit.

21. The method of claim 18, 19 or 20 producing arrays of fluid dots comprising providing an array of compliant deposit devices, the devices preferably being in the form of pin or pin-like structures, the devices having spacings corresponding to the well spacing of a 96 well plate, or a plate having a multiple of 96 wells or a spacing of 9 mm or a submultiple of 9 mm; preferably, according to a sampling plan, either dipping mobile annular supply rings into wells of the plate, or dipping the deposit devices directly into wells of the plate to provide fluid drops on the devices and transferring the drops to respective locations in substantially denser arrays on a receiving surface, preferably the drops being deposited on a microscope slide or a membrane supported on a slide-like member in a pattern of square arrays.

micron, in a dense array in a pattern corresponding to a function of the spacing of wells of a 96 well plate, the deposits preferably residing upon a glass microscope slide or on a fragile or soft surface, preferably a porous or microporous surface, the surface preferably comprising nitrocellulose, nylon, cellulose acetate, polyvinylidine fluoride or a gel, the fragile or soft surface preferably mounted on a rigid support.

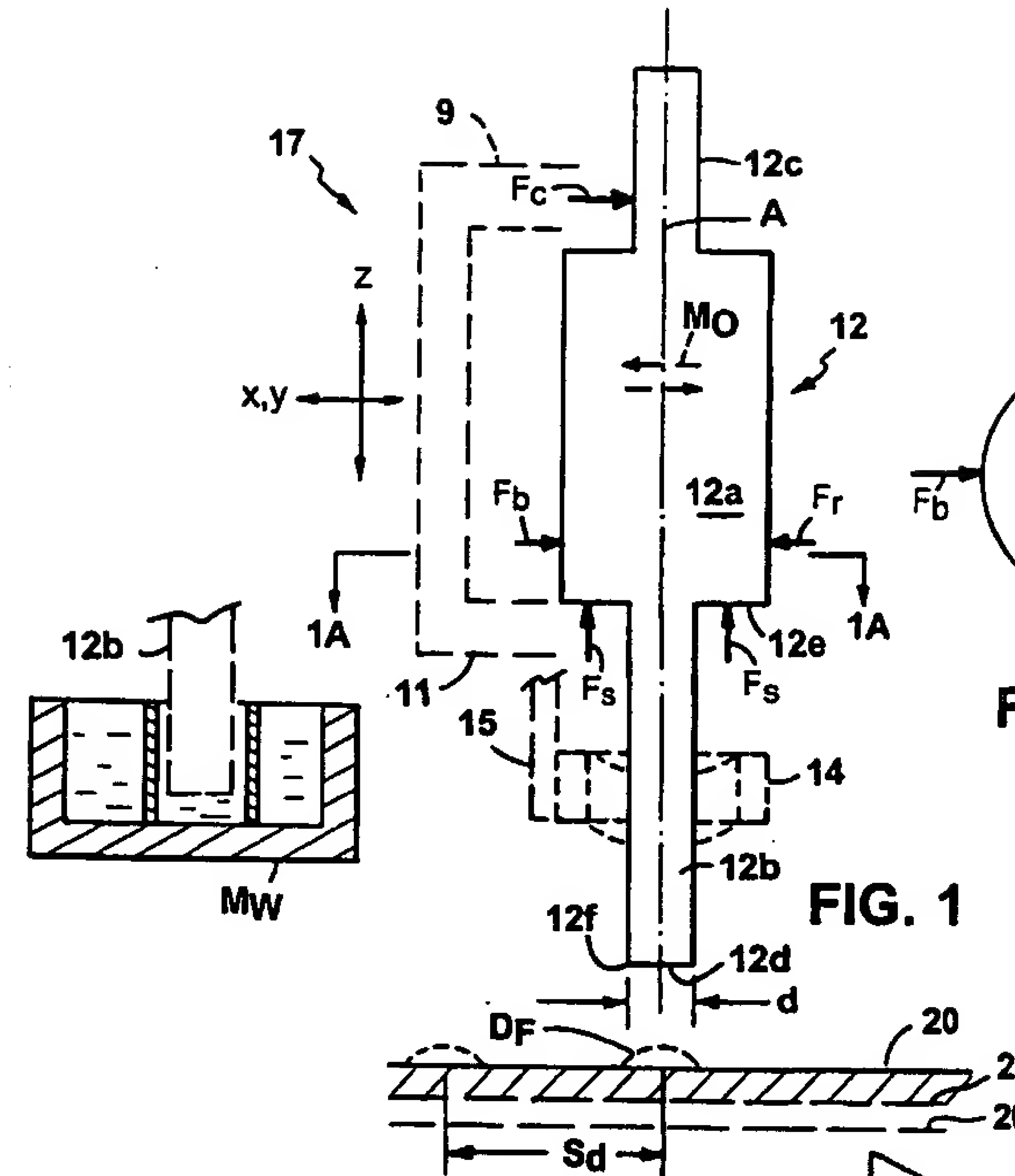


FIG. 1

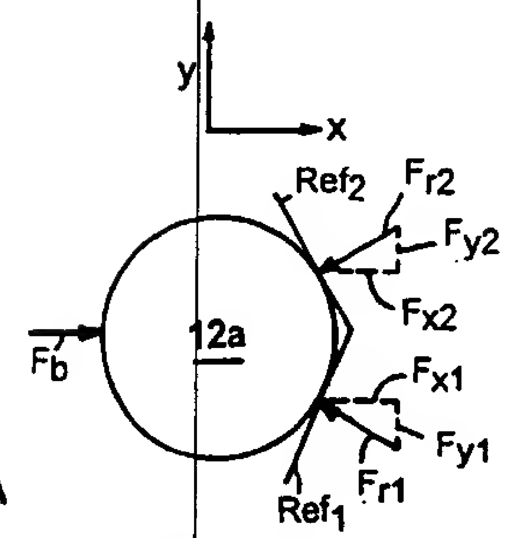


FIG. 1A

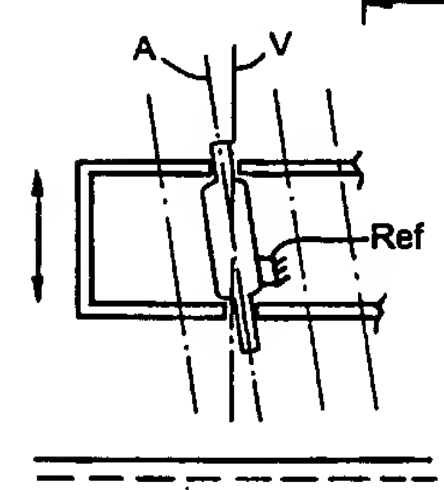


FIG. 1C

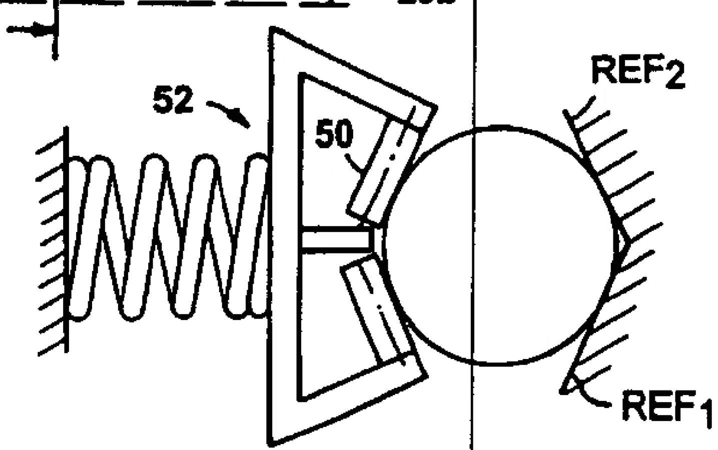


FIG. 1B

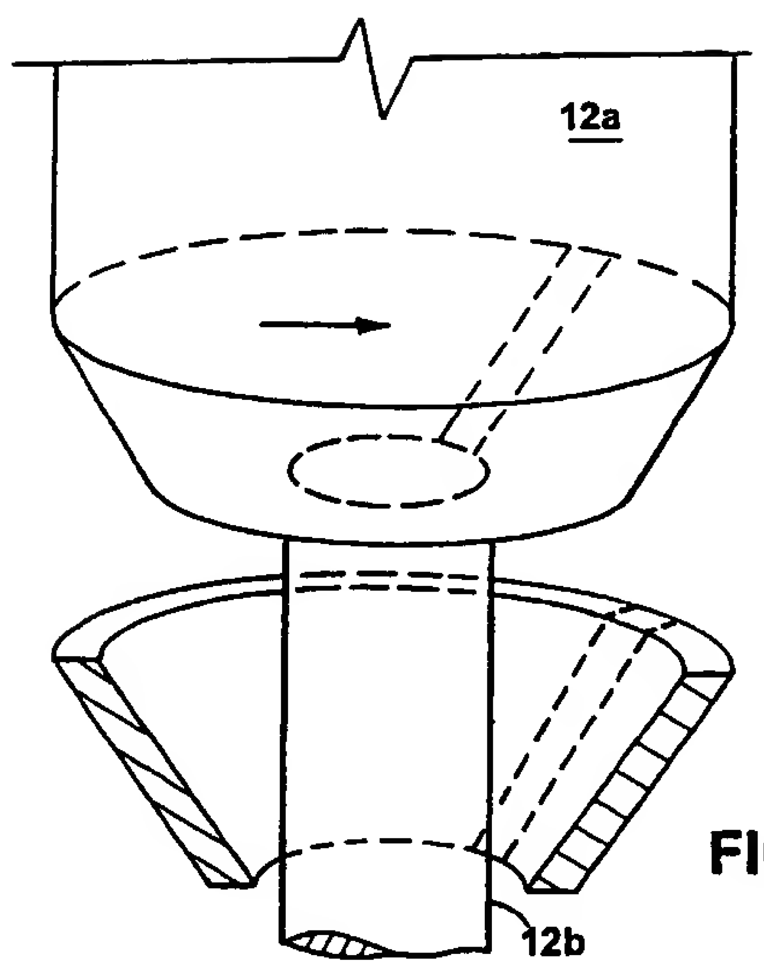


FIG. 1D

FIG. 1E

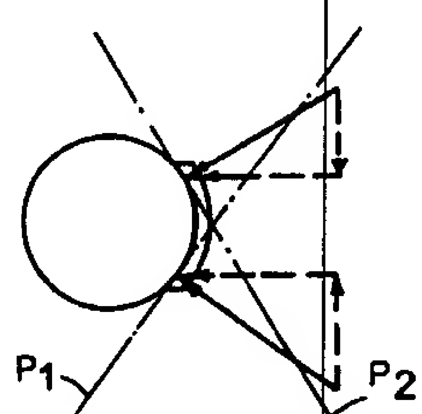


FIG. 1F

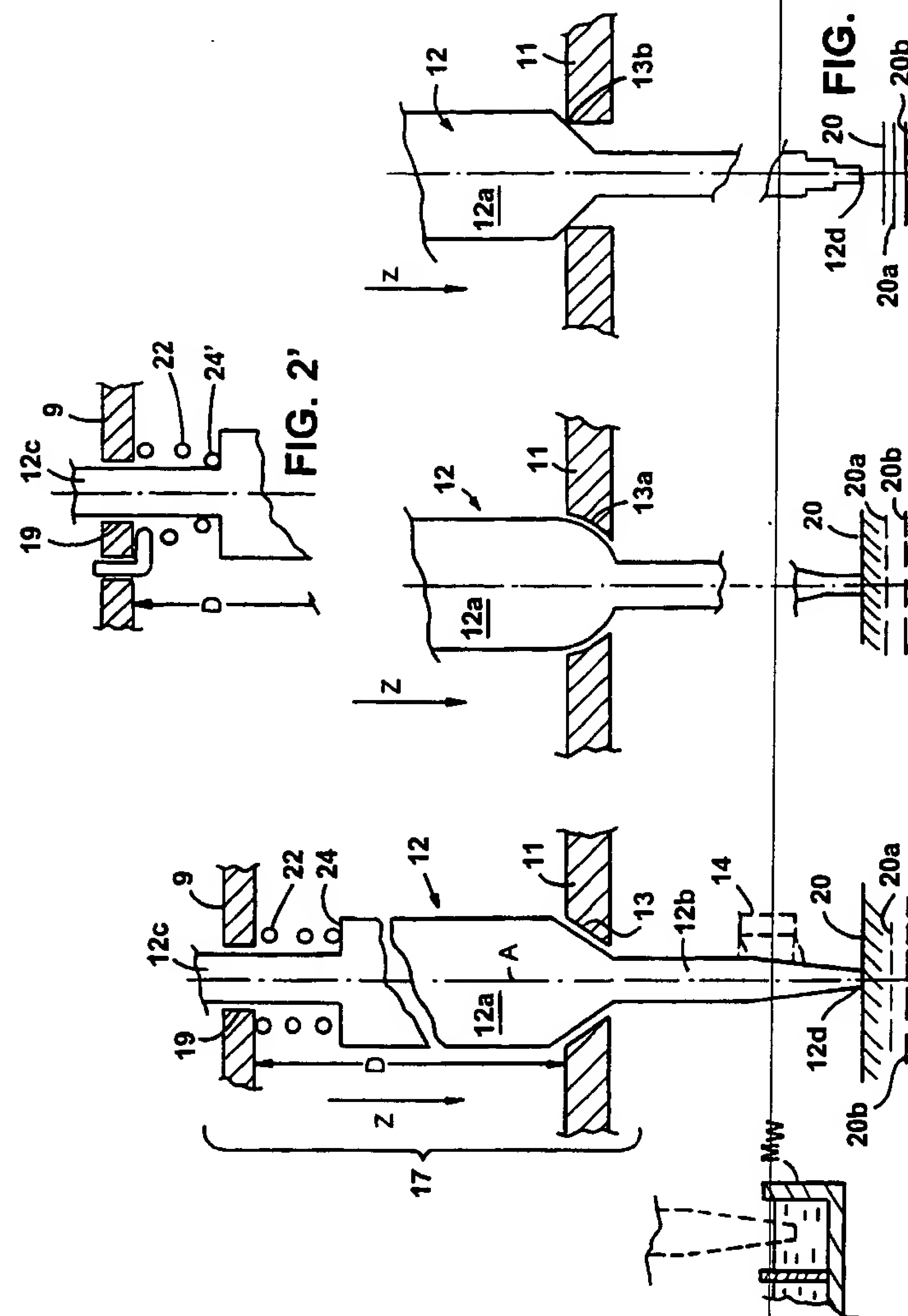


FIG. 2

FIG. 2'

FIG. 2A

FIG. 2B

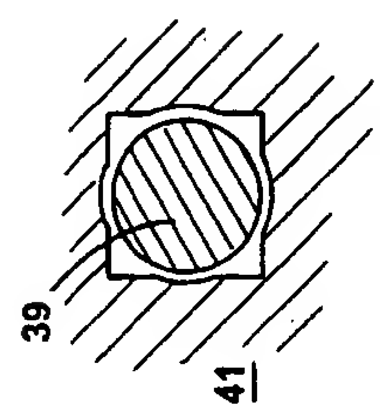


FIG. 3A

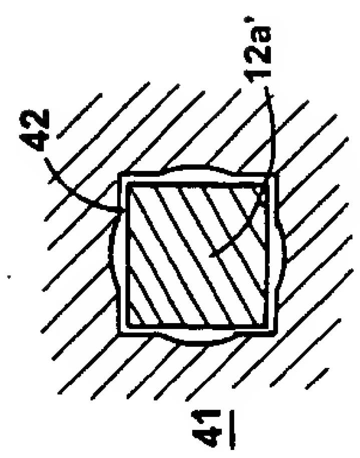


FIG. 3B

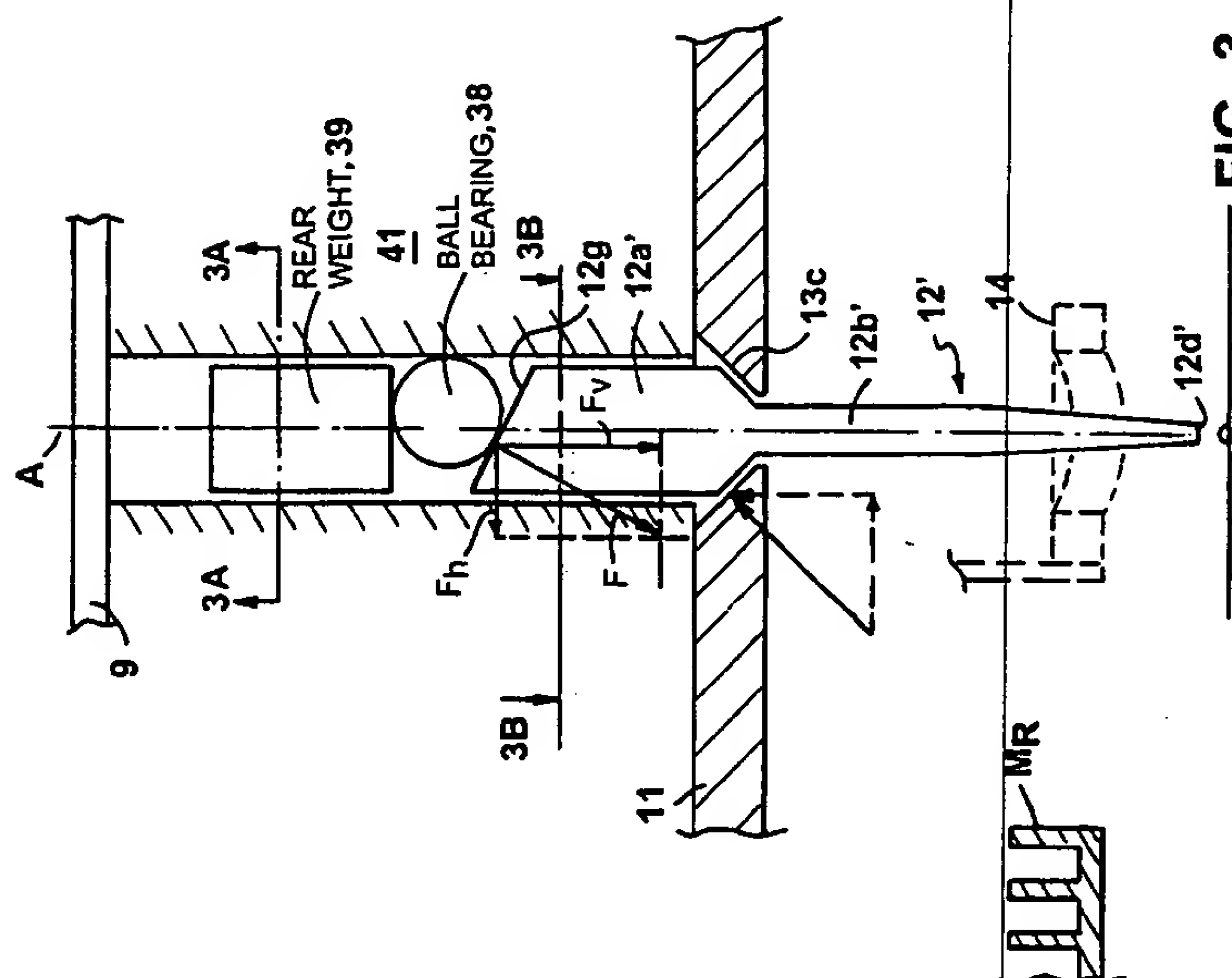


FIG. 3

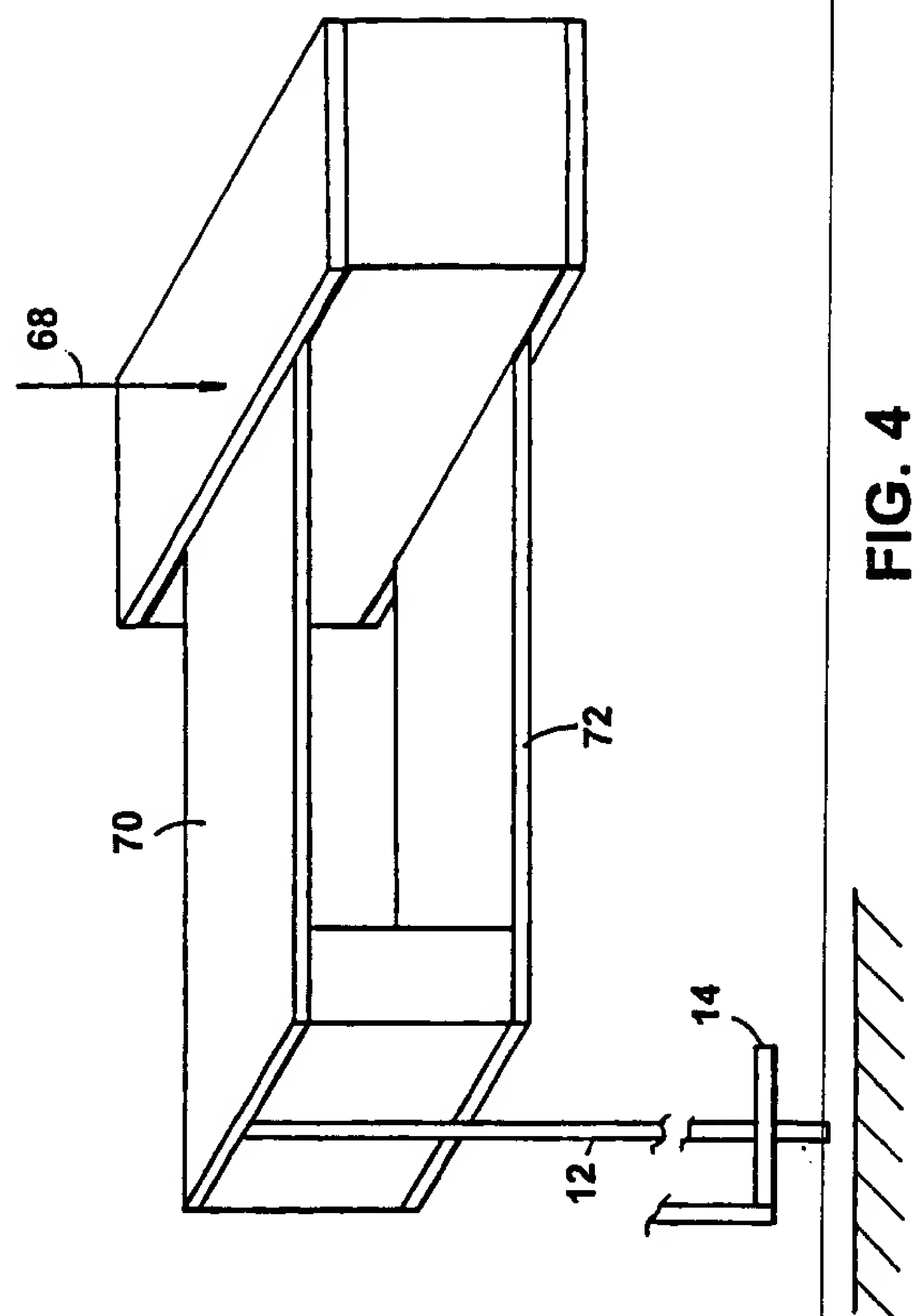


FIG. 4

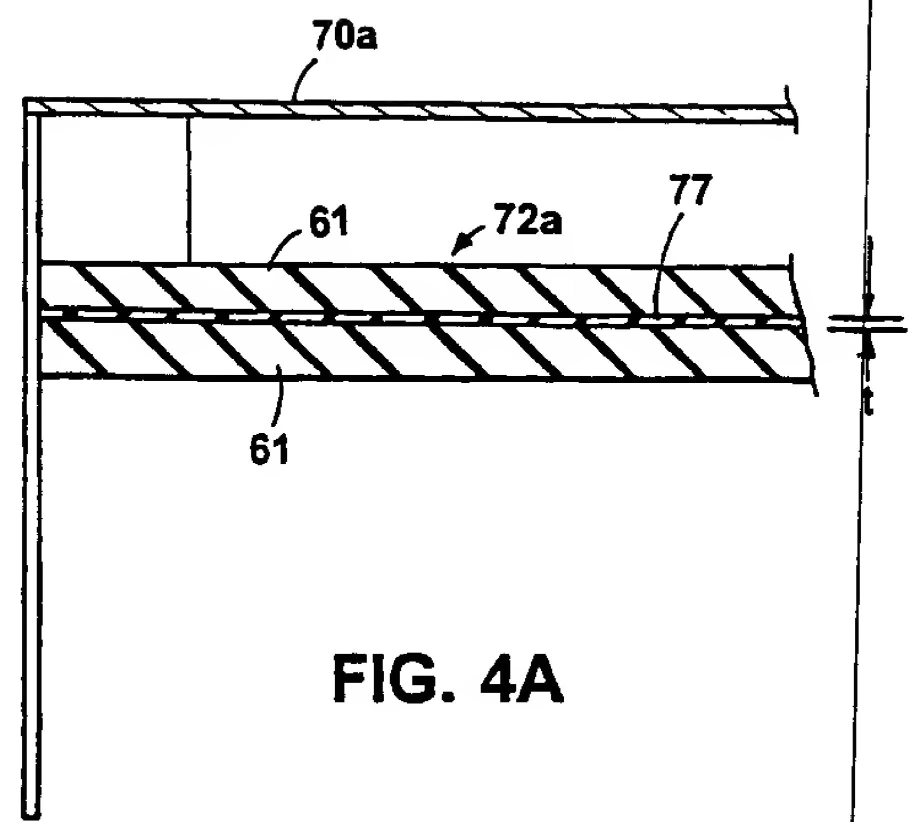


FIG. 4A

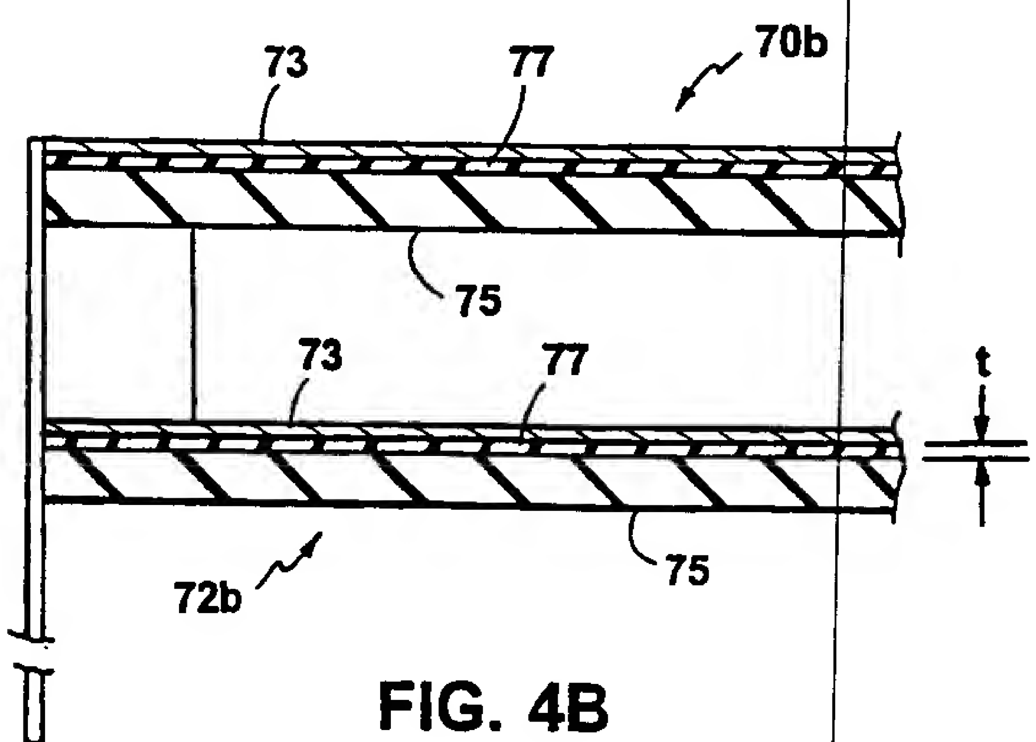


FIG. 4B

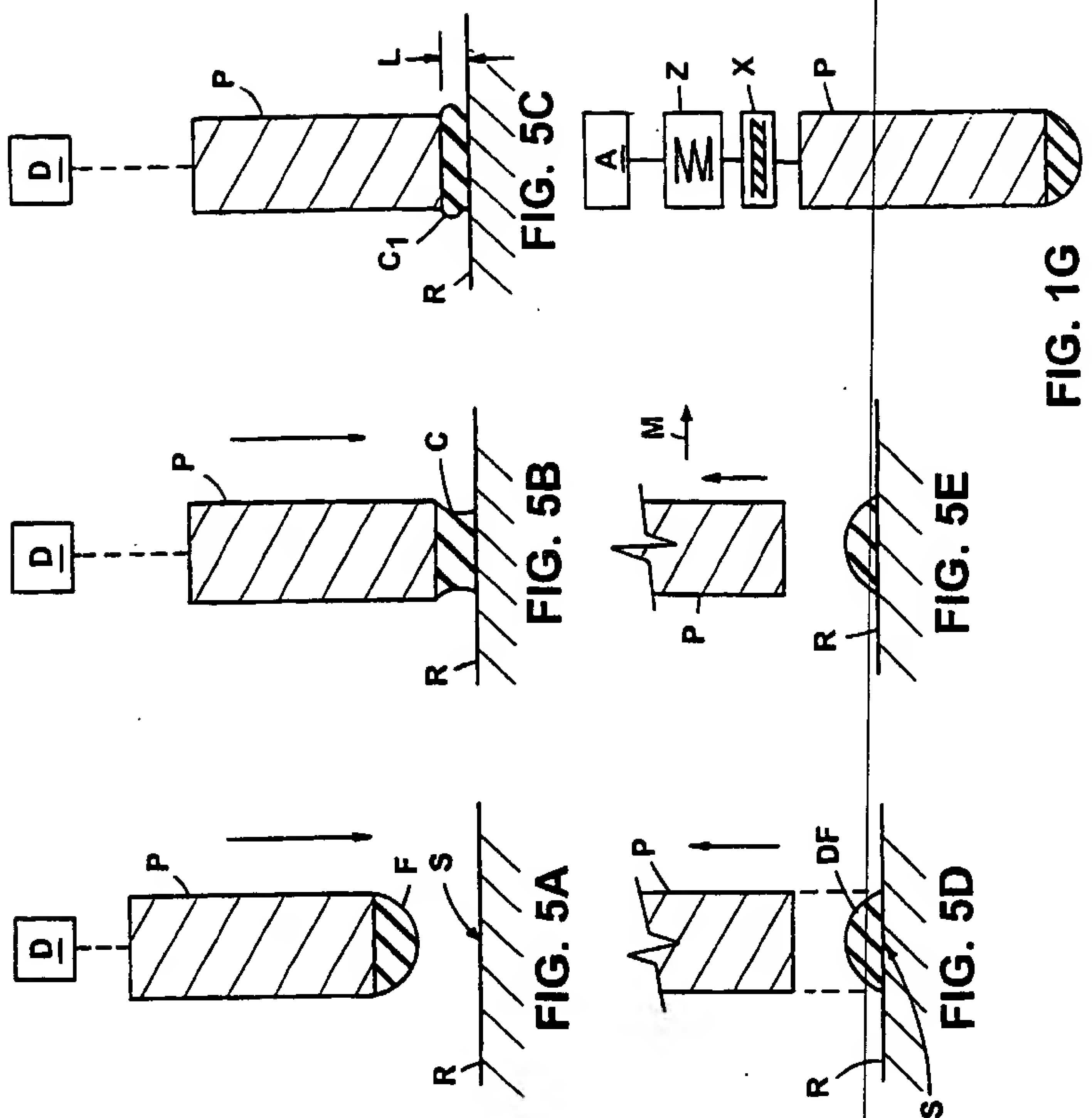


FIG. 5A

FIG. 5B

FIG. 5C

FIG. 5D

FIG. 5E

FIG. 1G

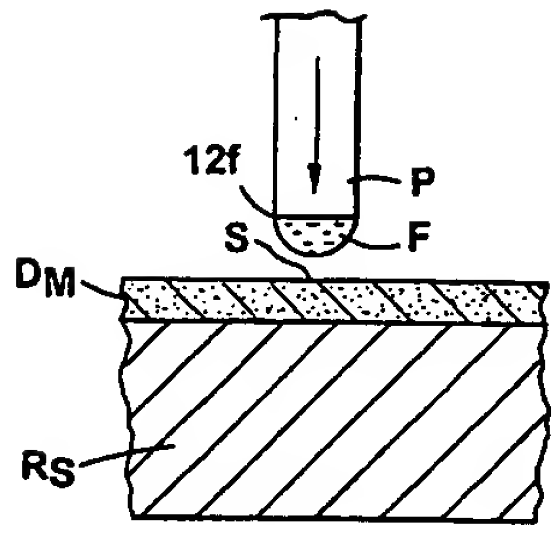


FIG. 5F

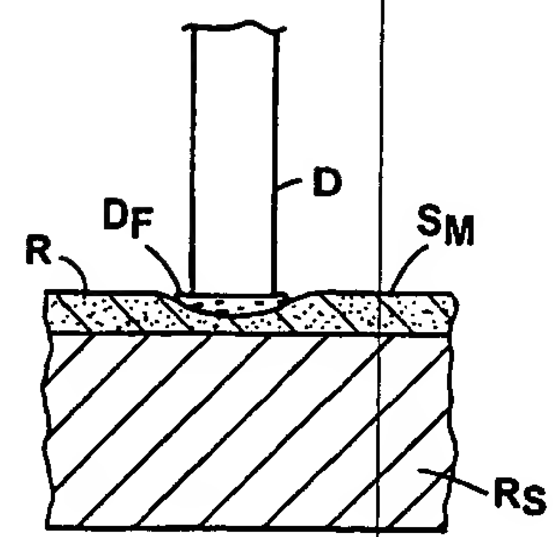


FIG. 5G

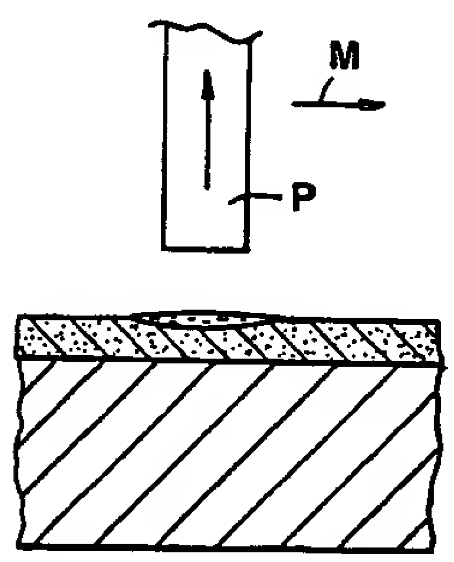


FIG. 5H

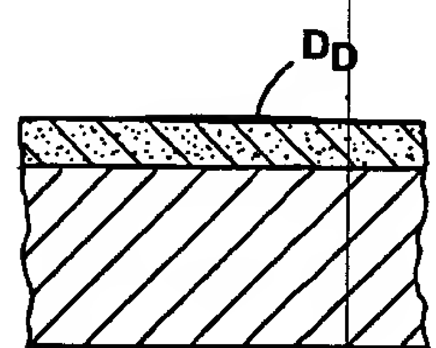


FIG. 5I

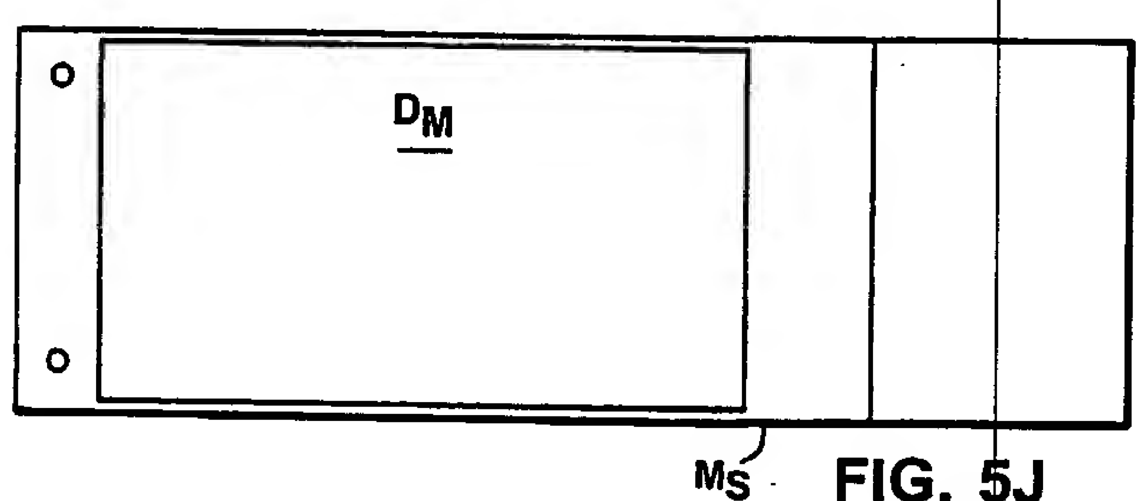


FIG. 5J

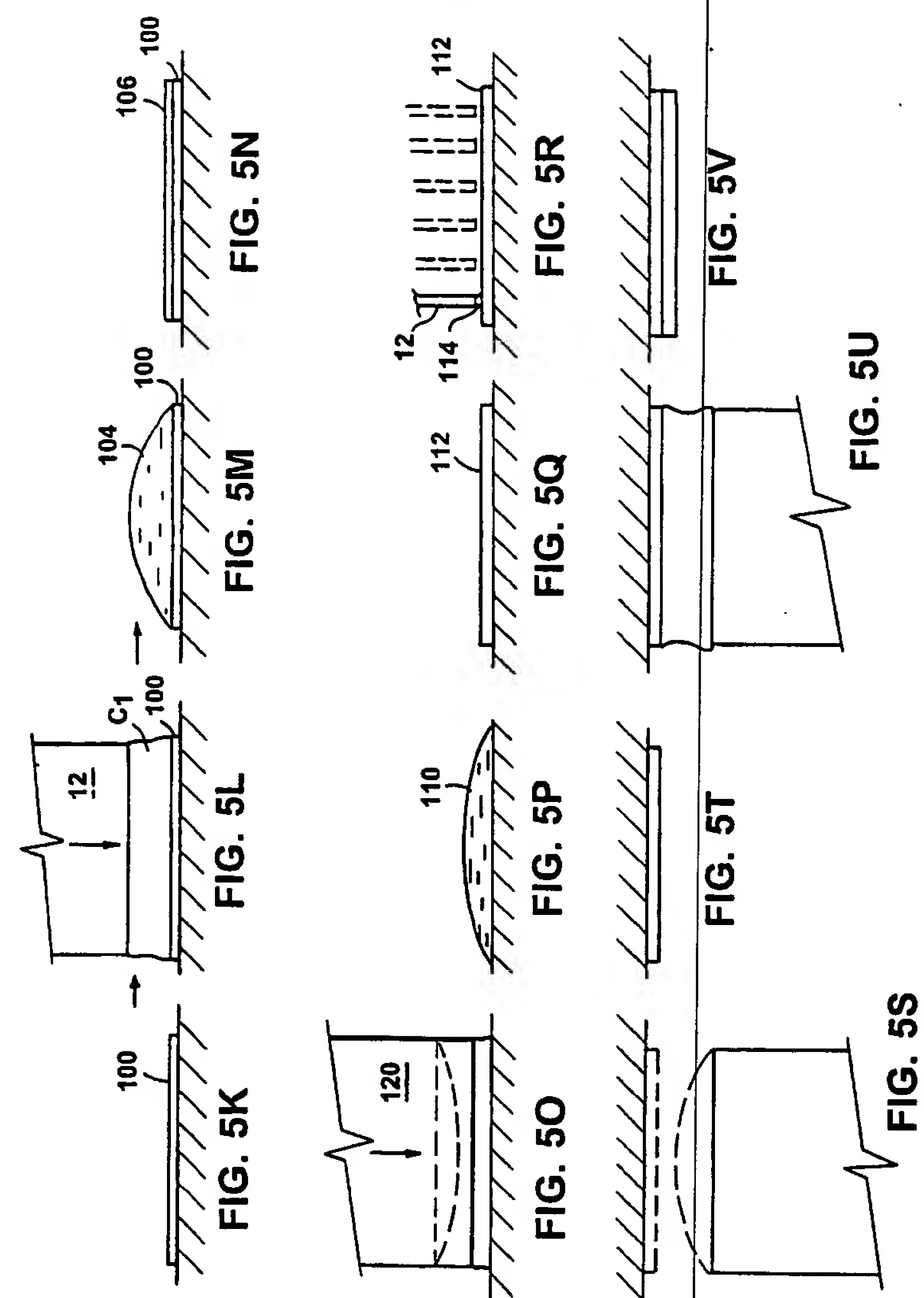


FIG. 5K FIG. 5L FIG. 5M FIG. 5N

FIG. 5O FIG. 5P FIG. 5Q FIG. 5R

FIG. 5S FIG. 5T FIG. 5U FIG. 5V

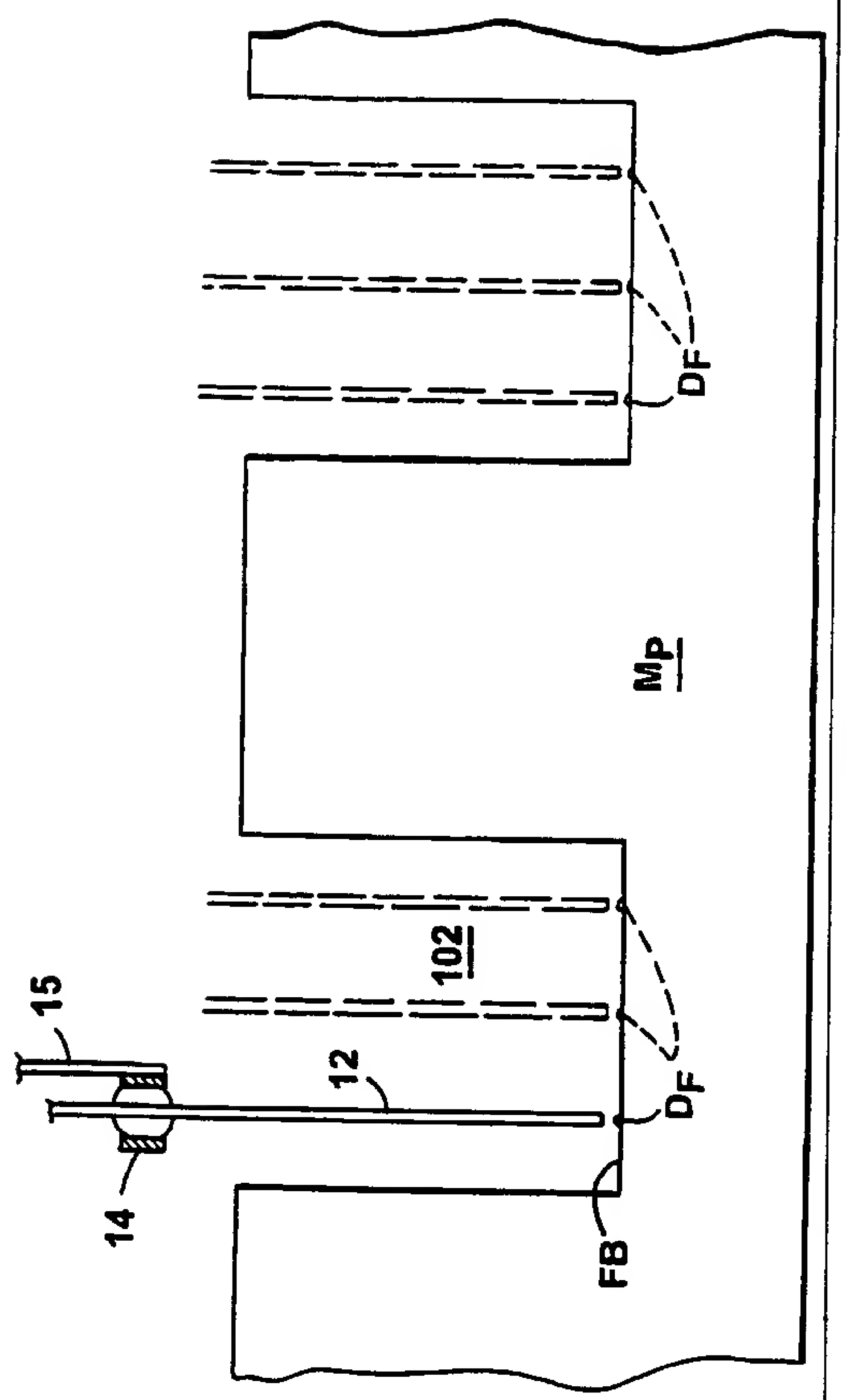


FIG. 6

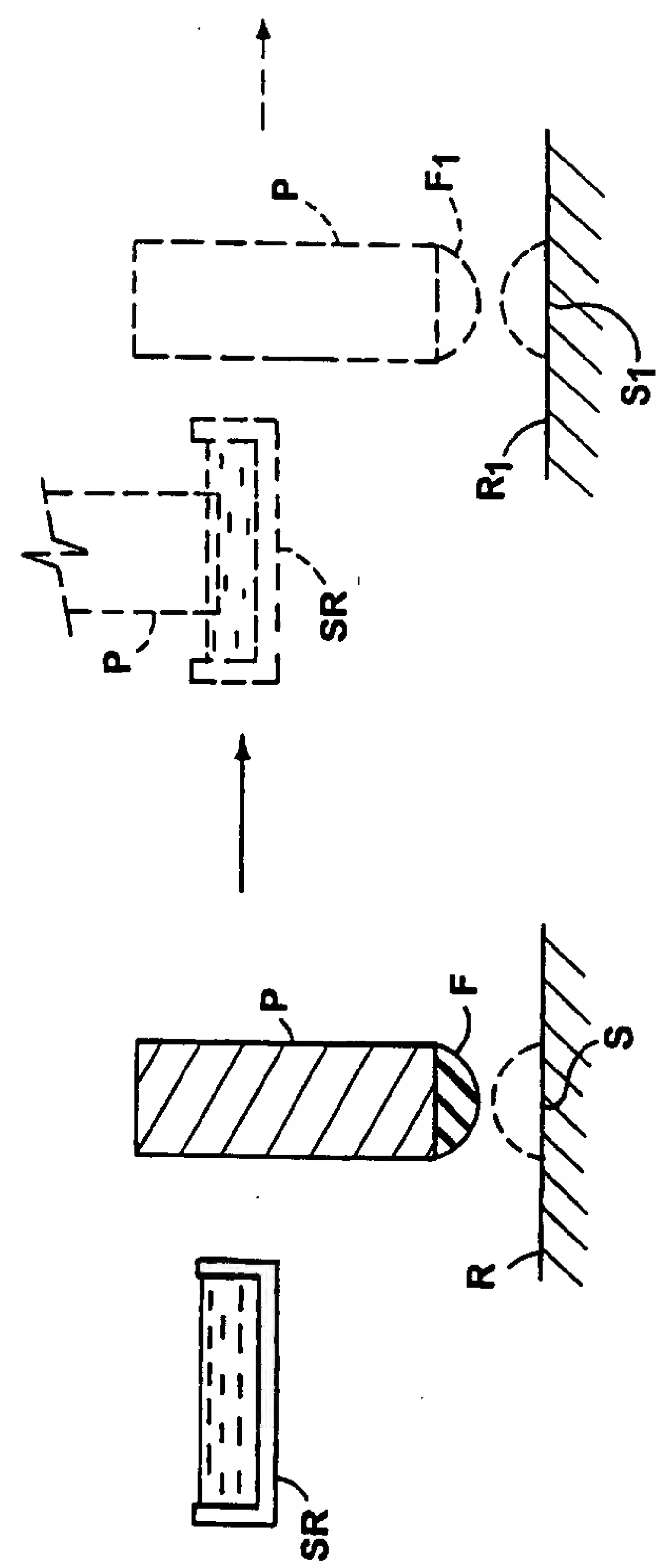


FIG. 7

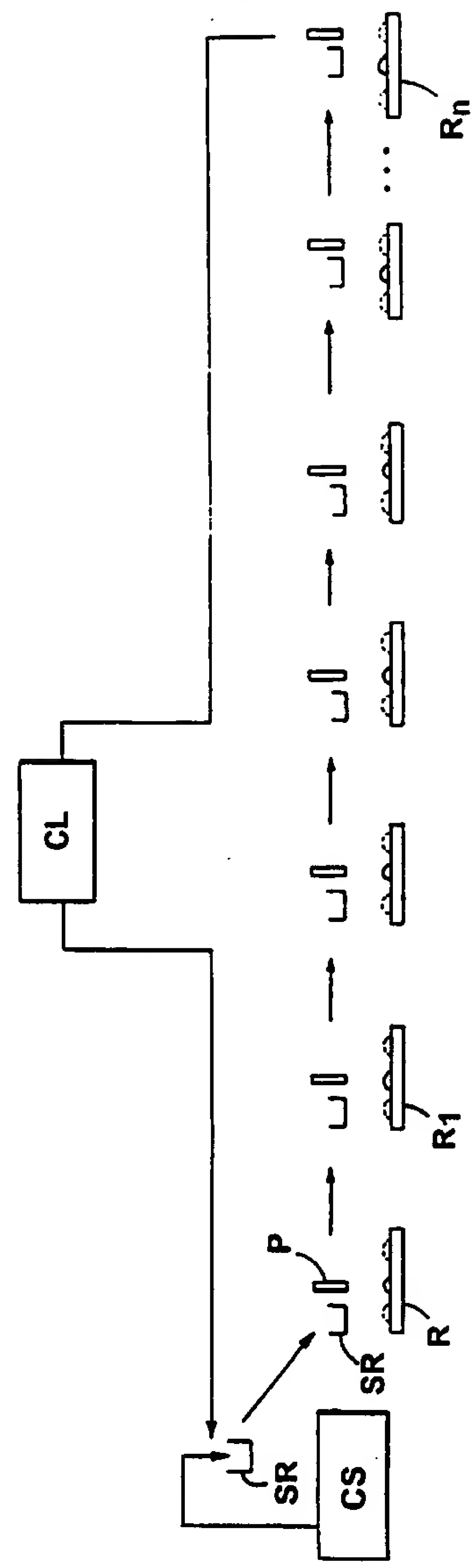


FIG. 8

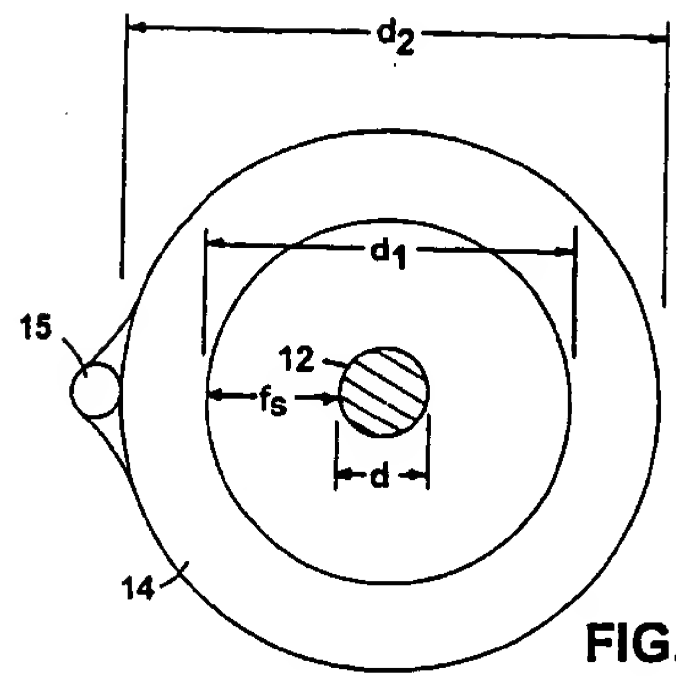


FIG. 10

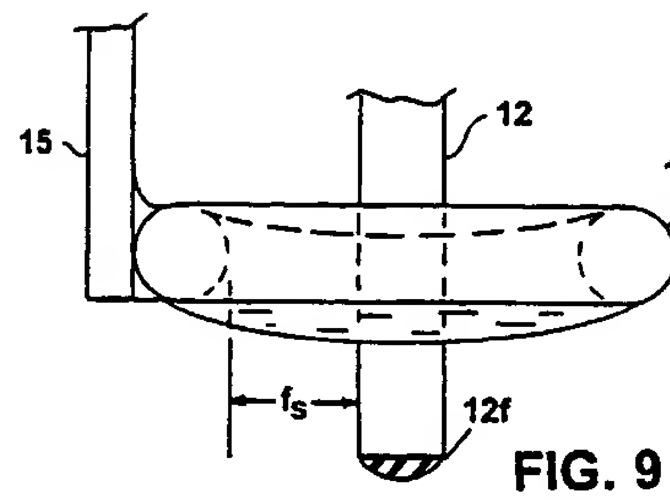


FIG. 9

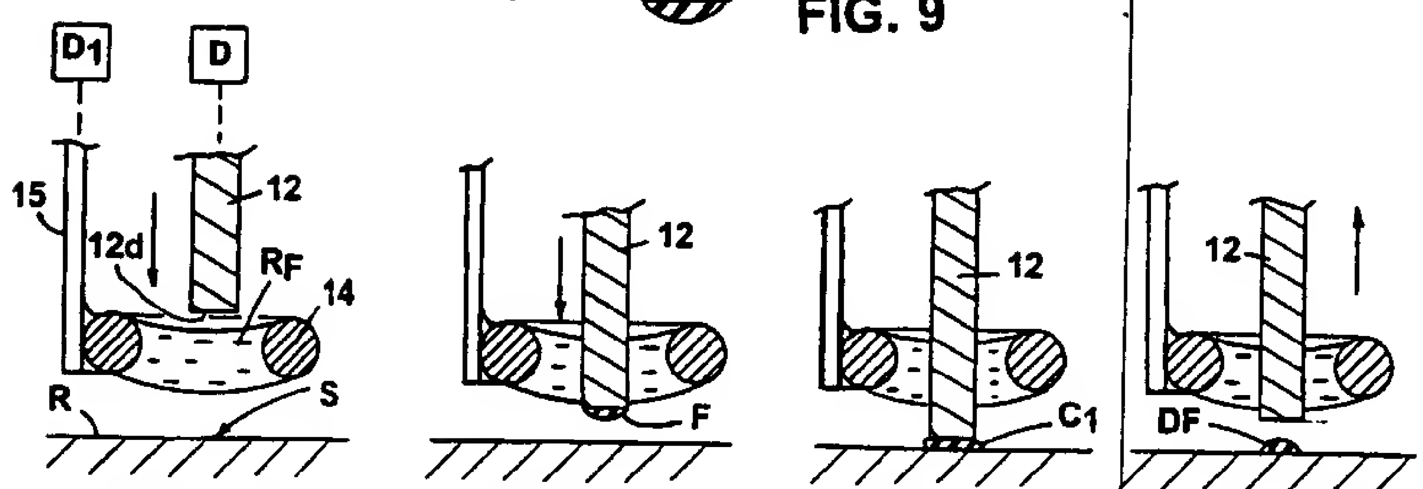


FIG. 9A FIG. 9B FIG. 9C FIG. 9D

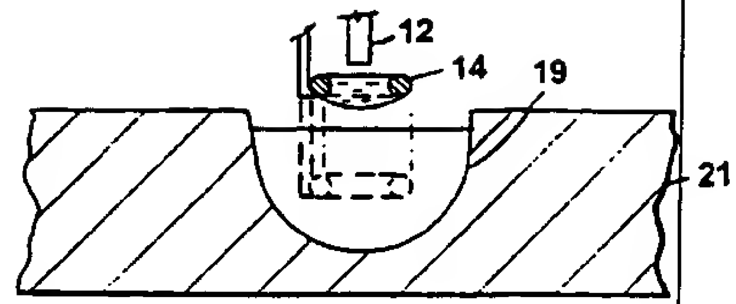


FIG. 9E

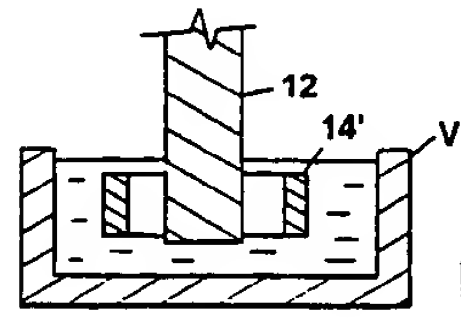


FIG. 9F

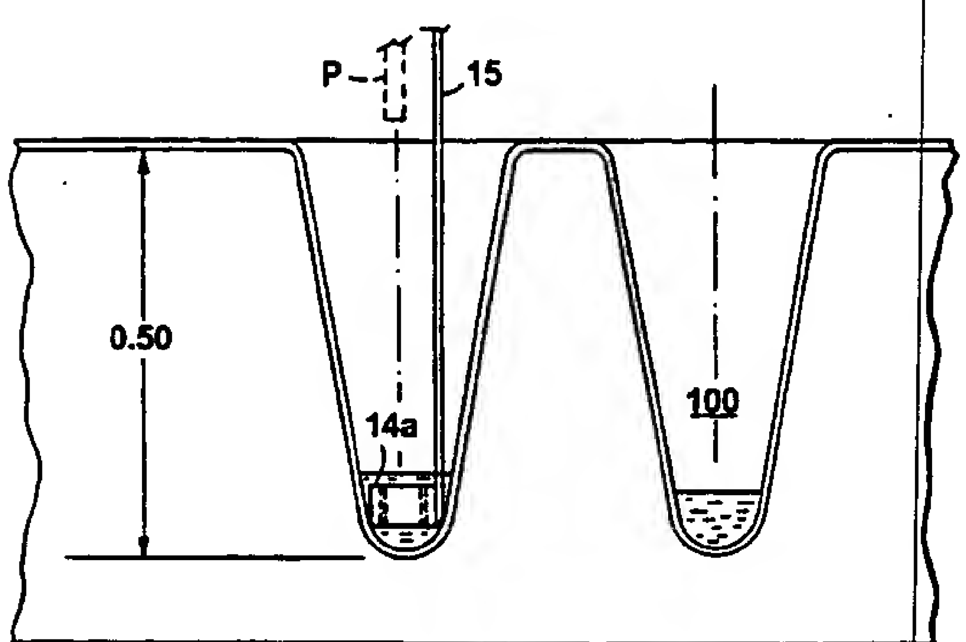


FIG. 9H

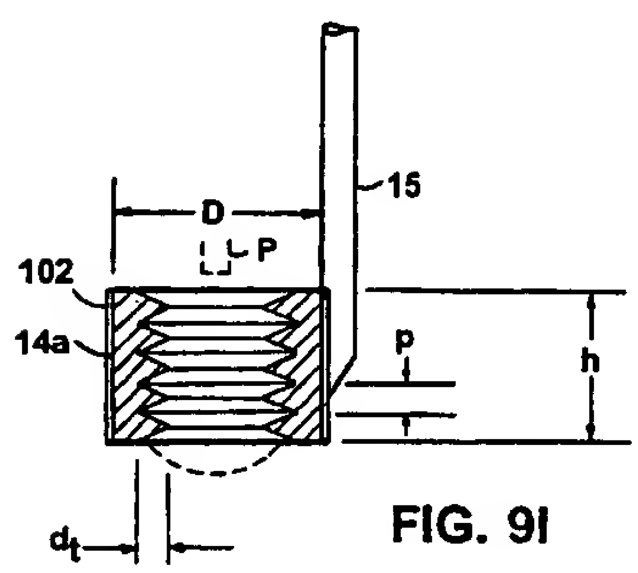


FIG. 9I

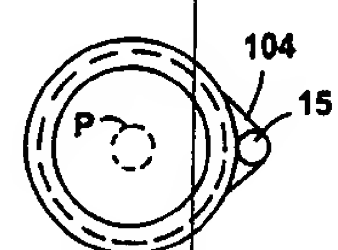


FIG. 9J

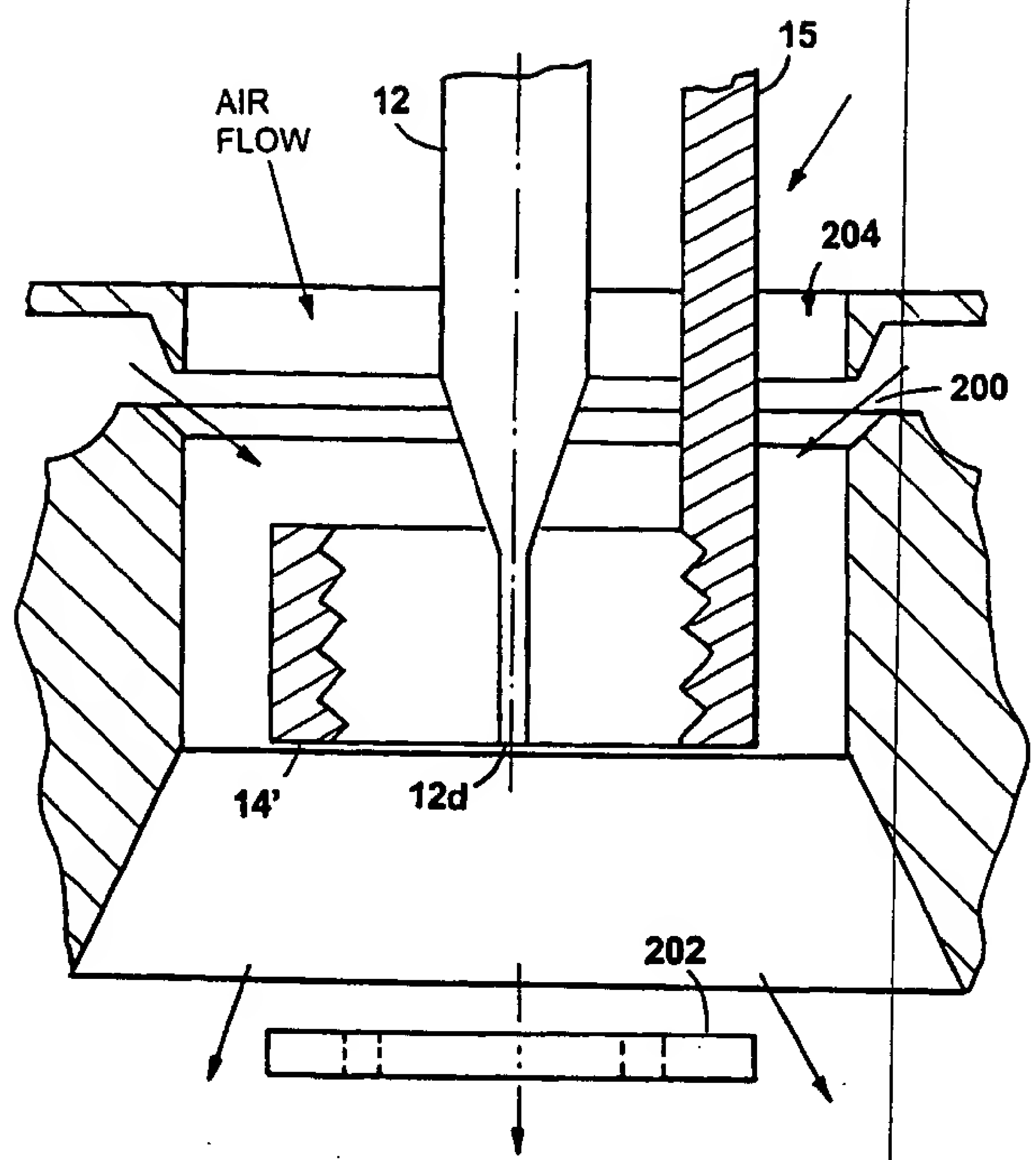
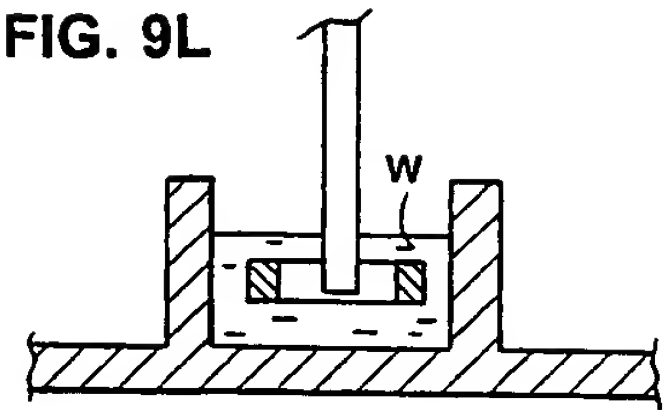


FIG. 9G

FIG. 9L



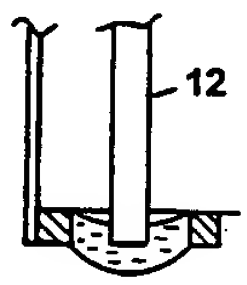


FIG. 9M

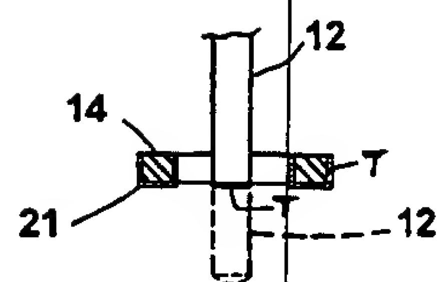


FIG. 9K

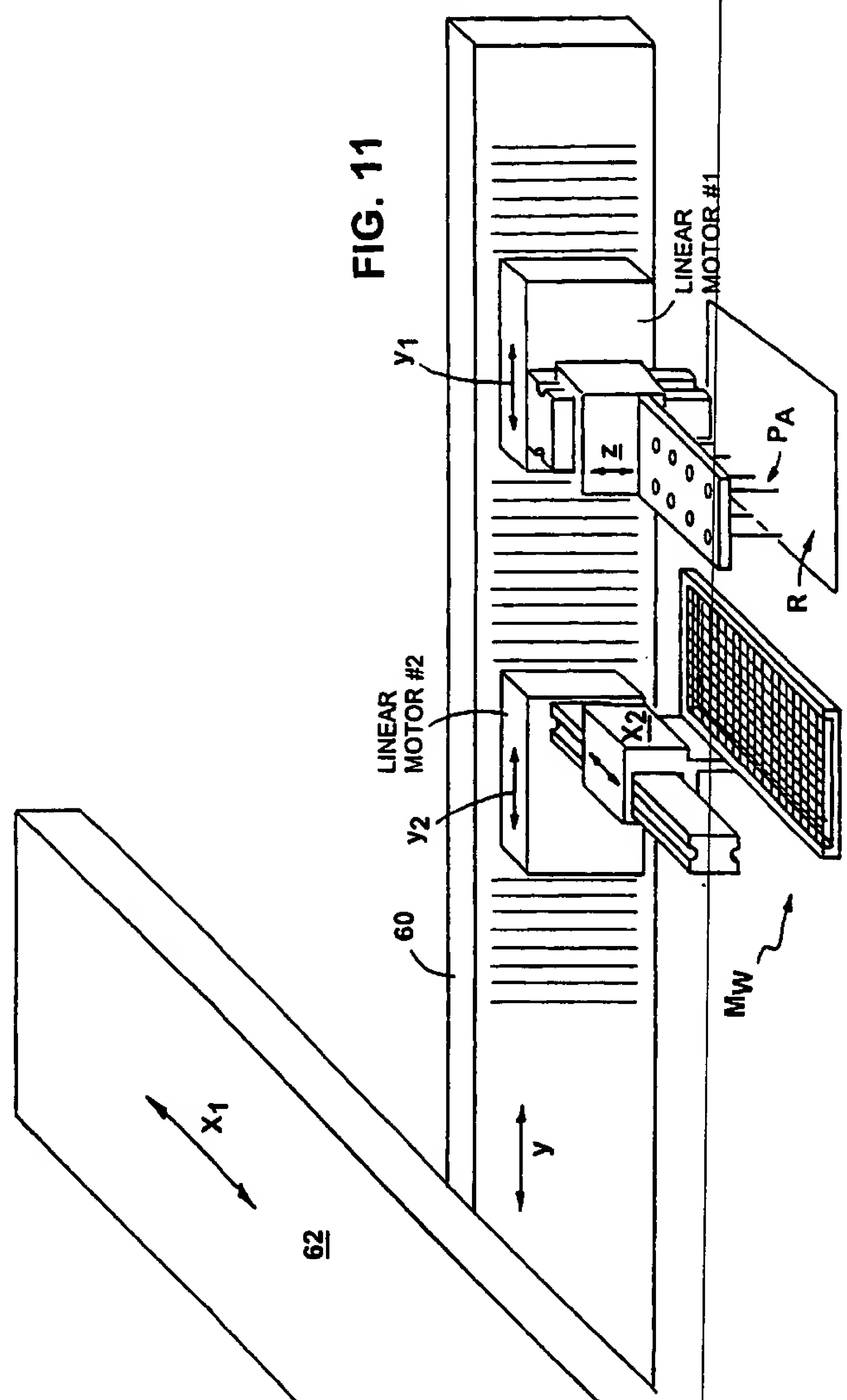


FIG. 11

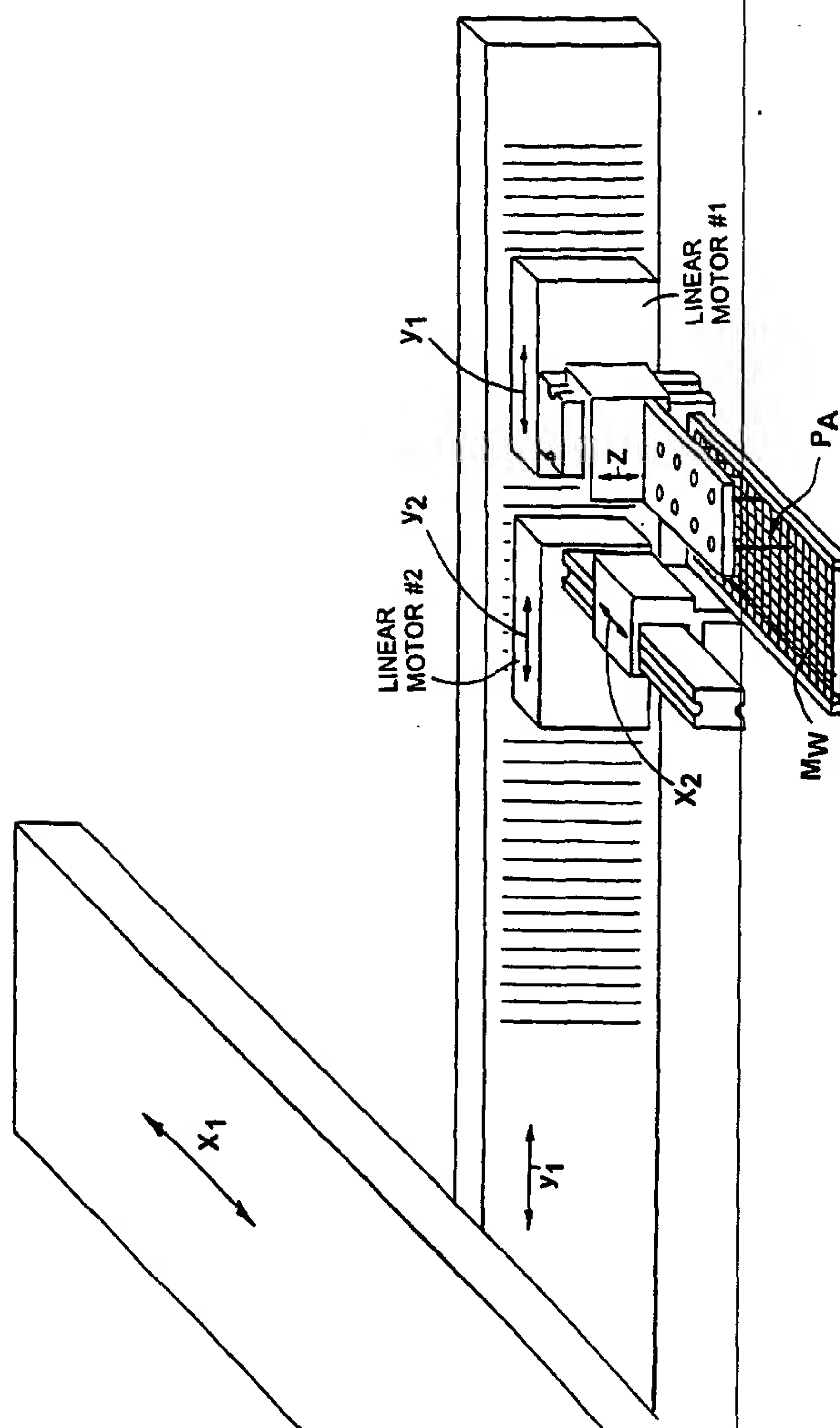


FIG. 11A

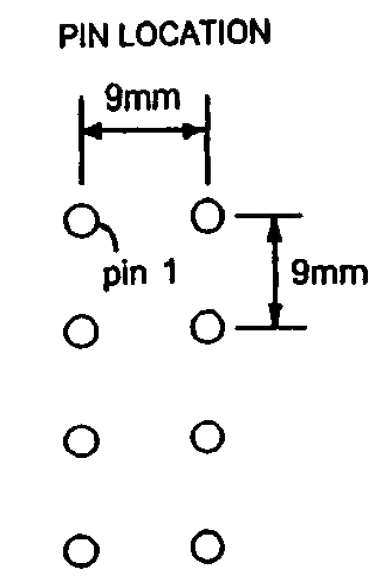


FIG. 12

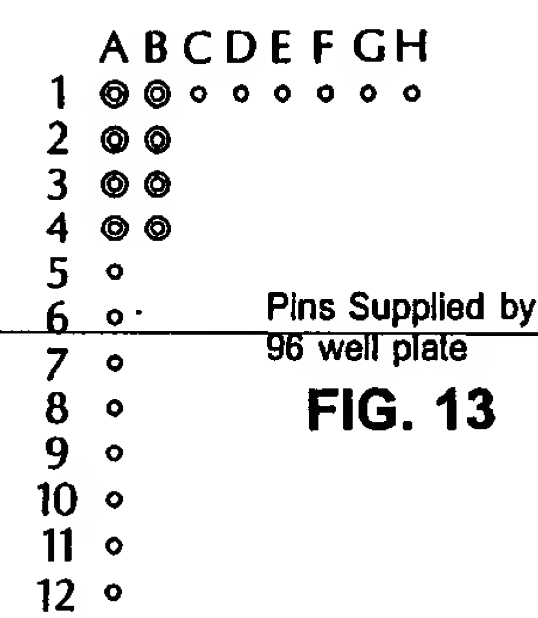


FIG. 13

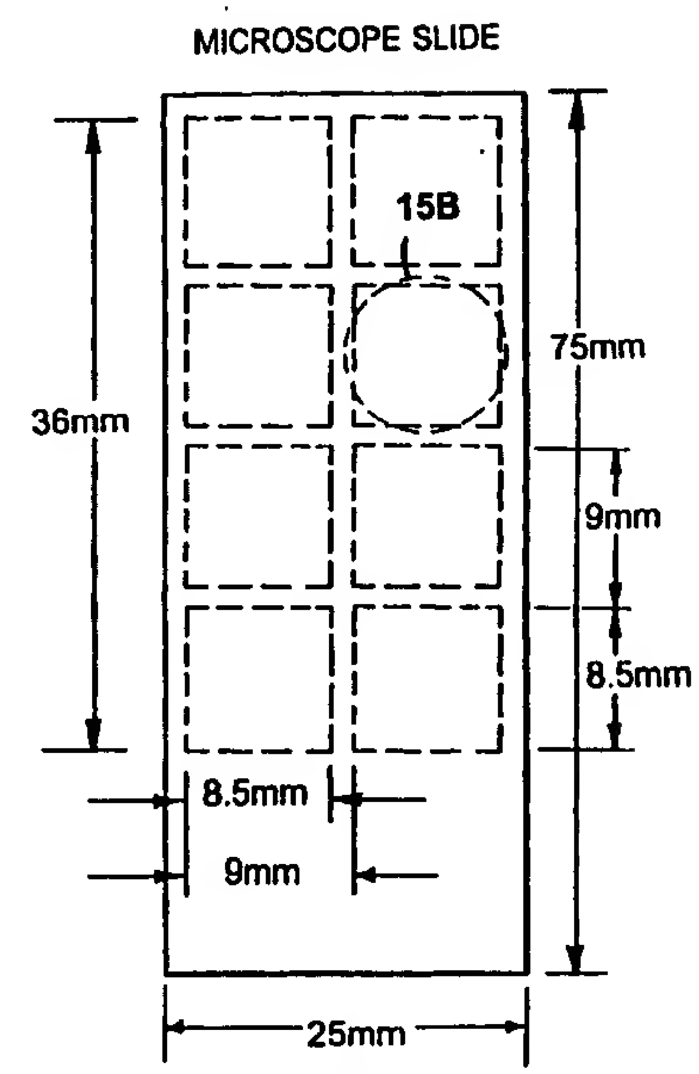


FIG. 15

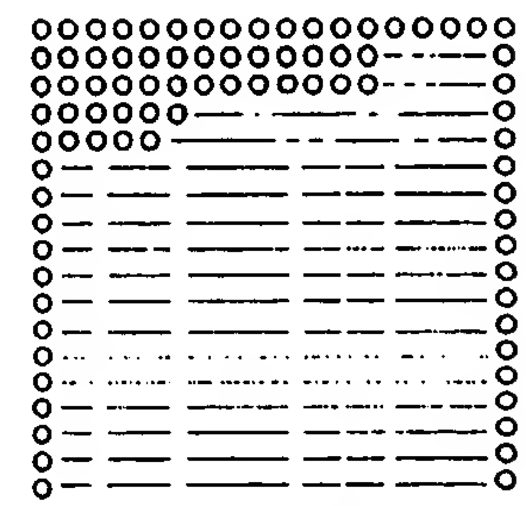


FIG. 15B

The pin assembly follows the pattern for supply:

pin 1 →	R1	COL A
	R1	COL C
	R1	COL E
	R1	COL G
	R5	COL A
	R5	COL C
	R5	COL E
	R5	COL G
	R9	COL A
	R9	COL C
	R9	COL E
	R9	COL G

FIG. 14

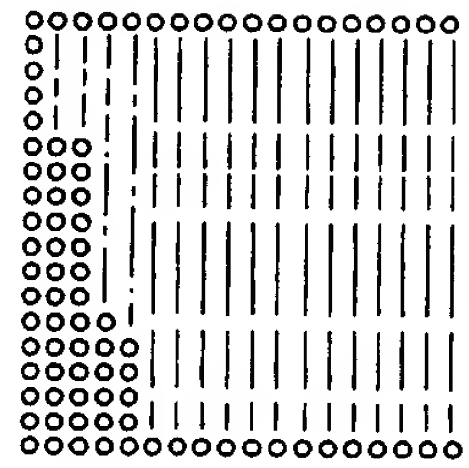


FIG. 19B

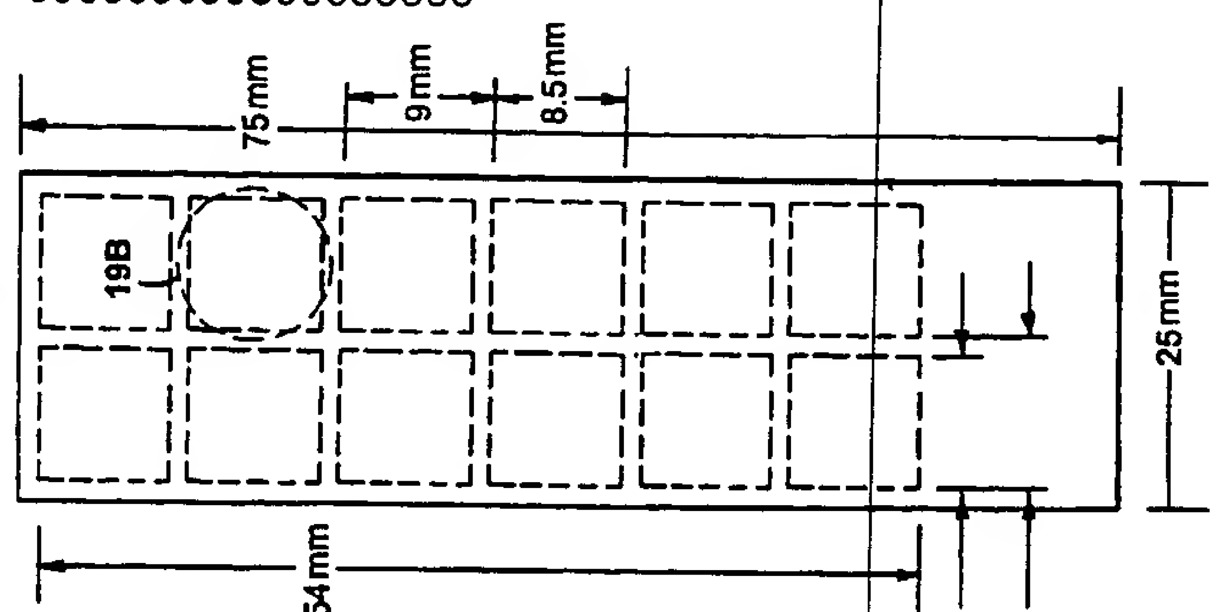


FIG. 19

The pin assembly follows the pattern

COL A ROW 1
COL C ROW 1
COL E ROW 1
COL G ROW 1
COL A ROW 7
COL C ROW 7
COL E ROW 7
COL G ROW 7

FIG. 18

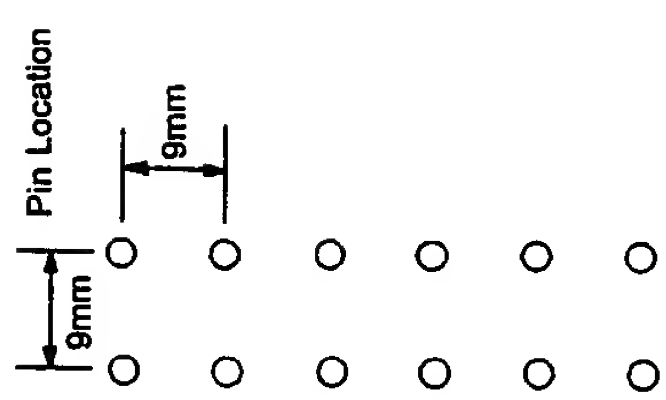


FIG. 16

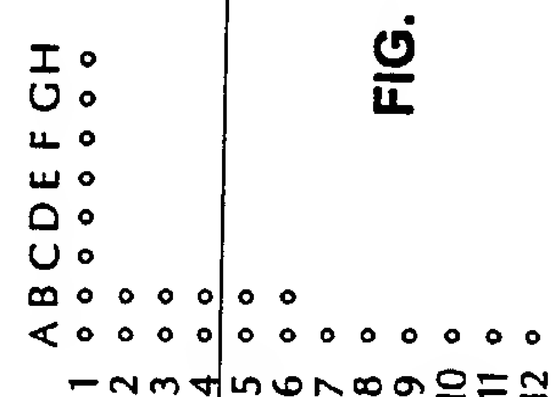


FIG. 17

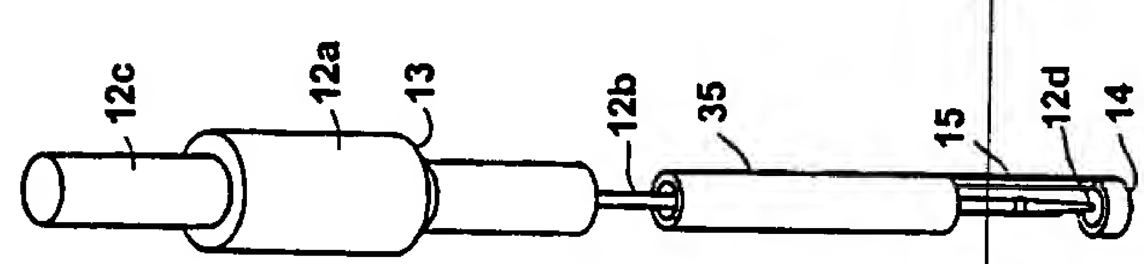


FIG. 20

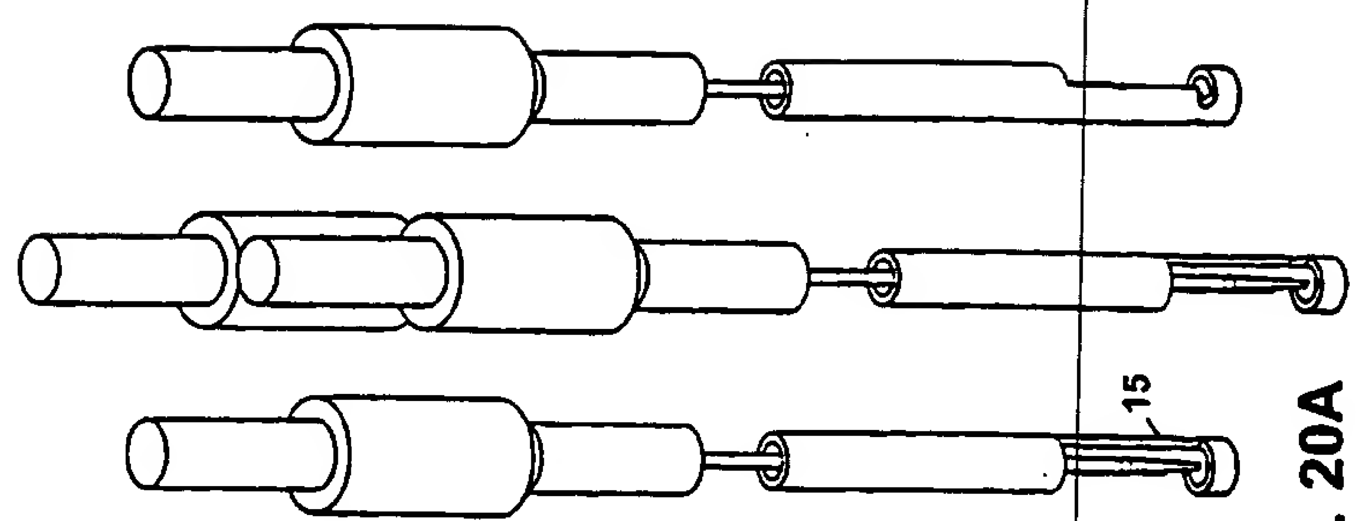


FIG. 20A

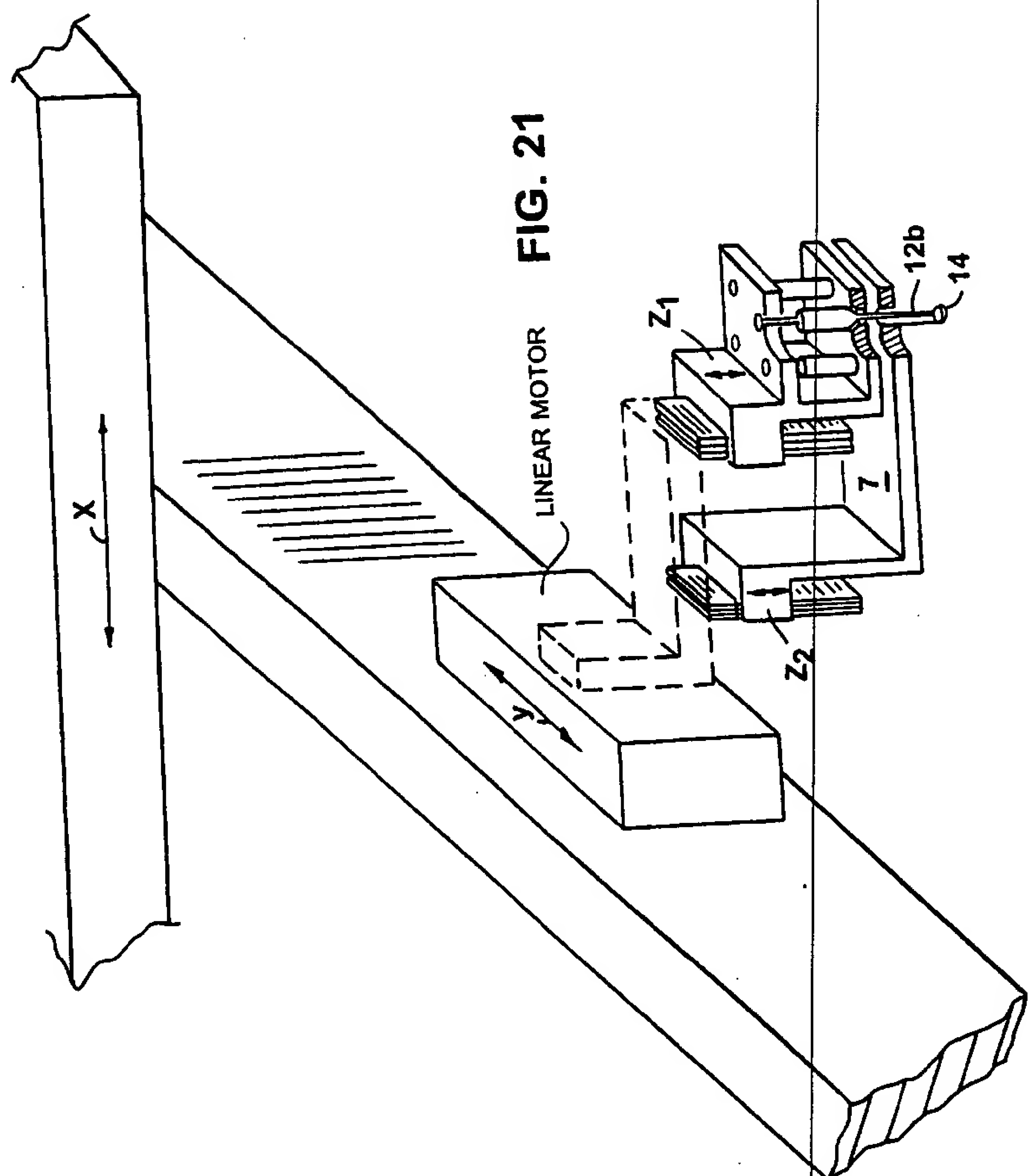
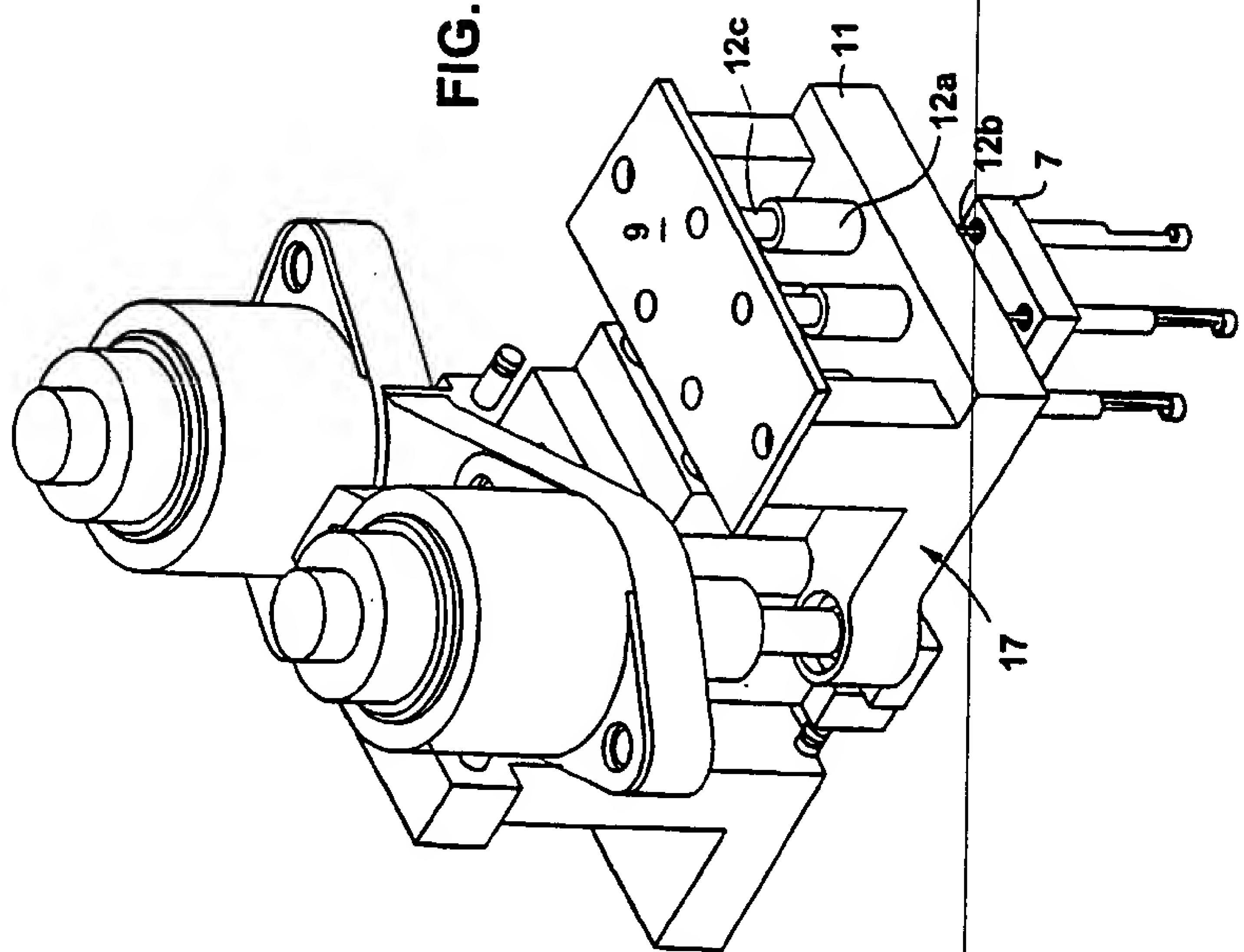


FIG. 21

FIG. 22



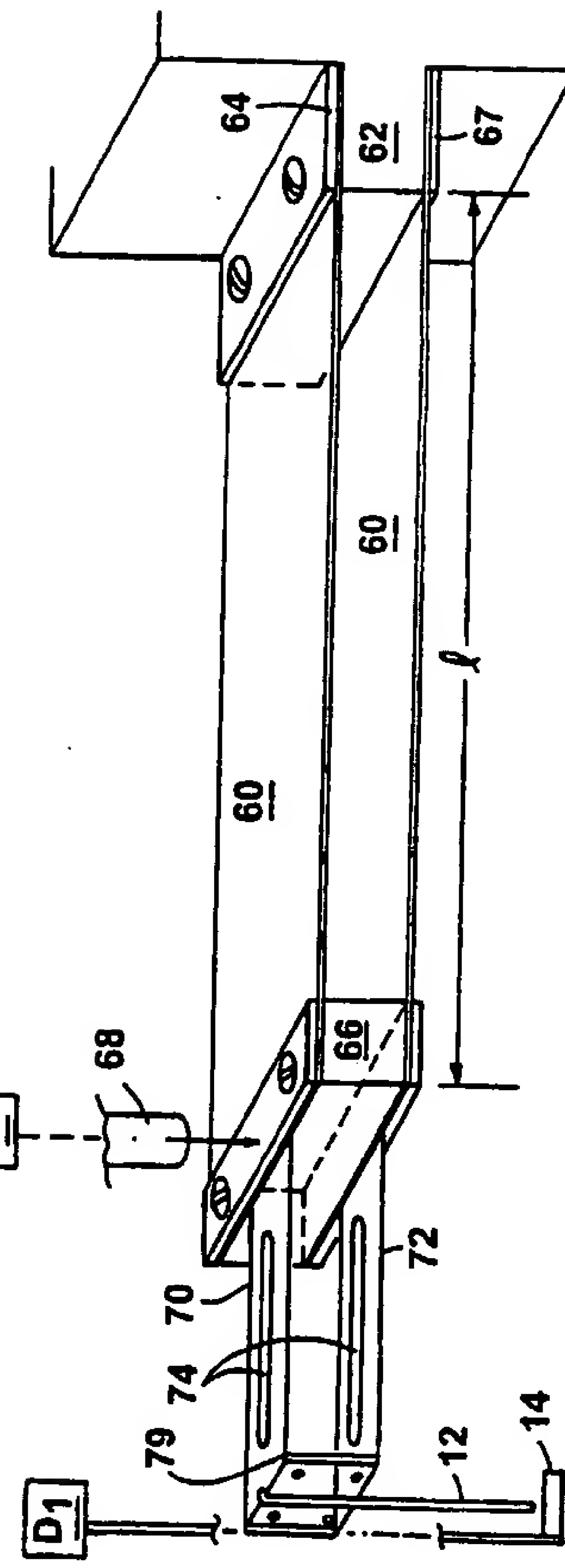


FIG. 23

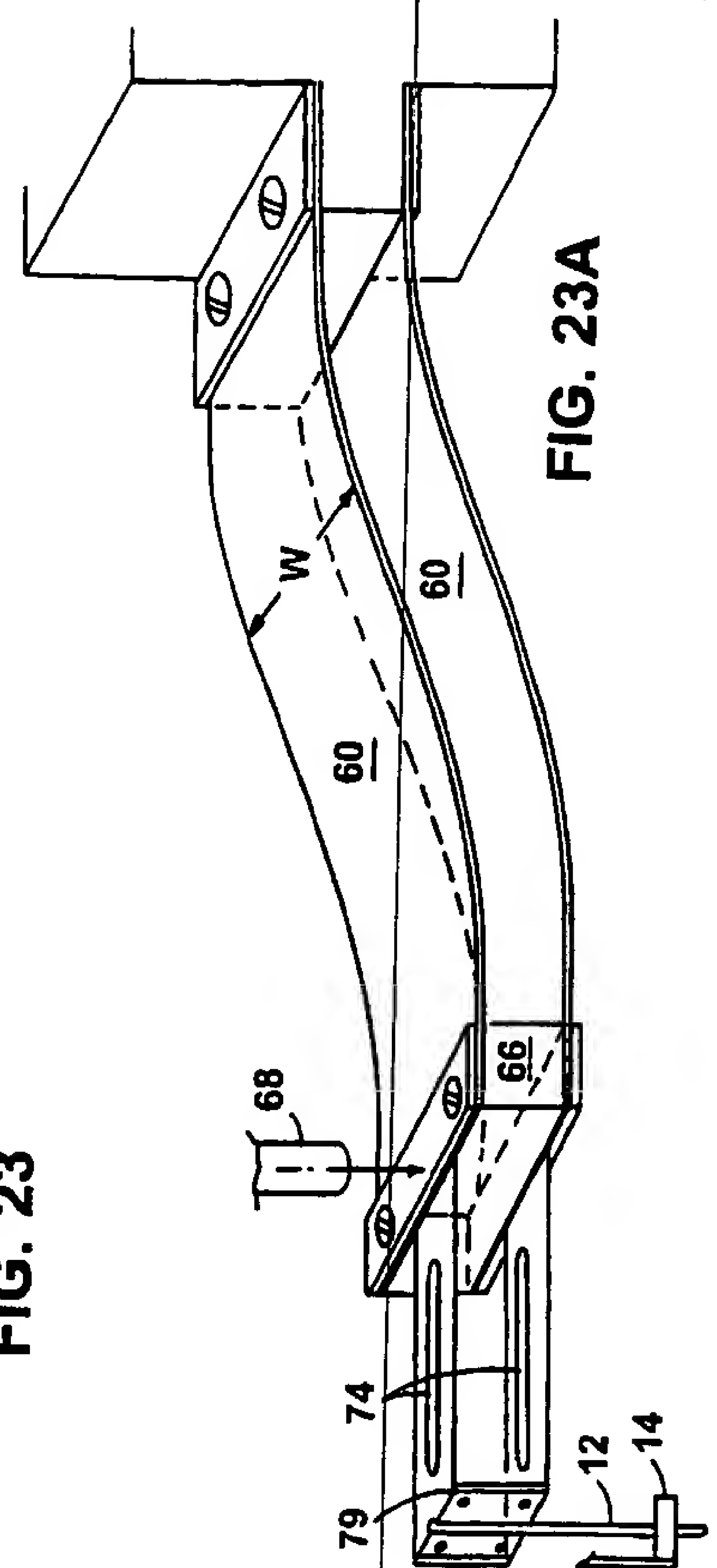


FIG. 23A

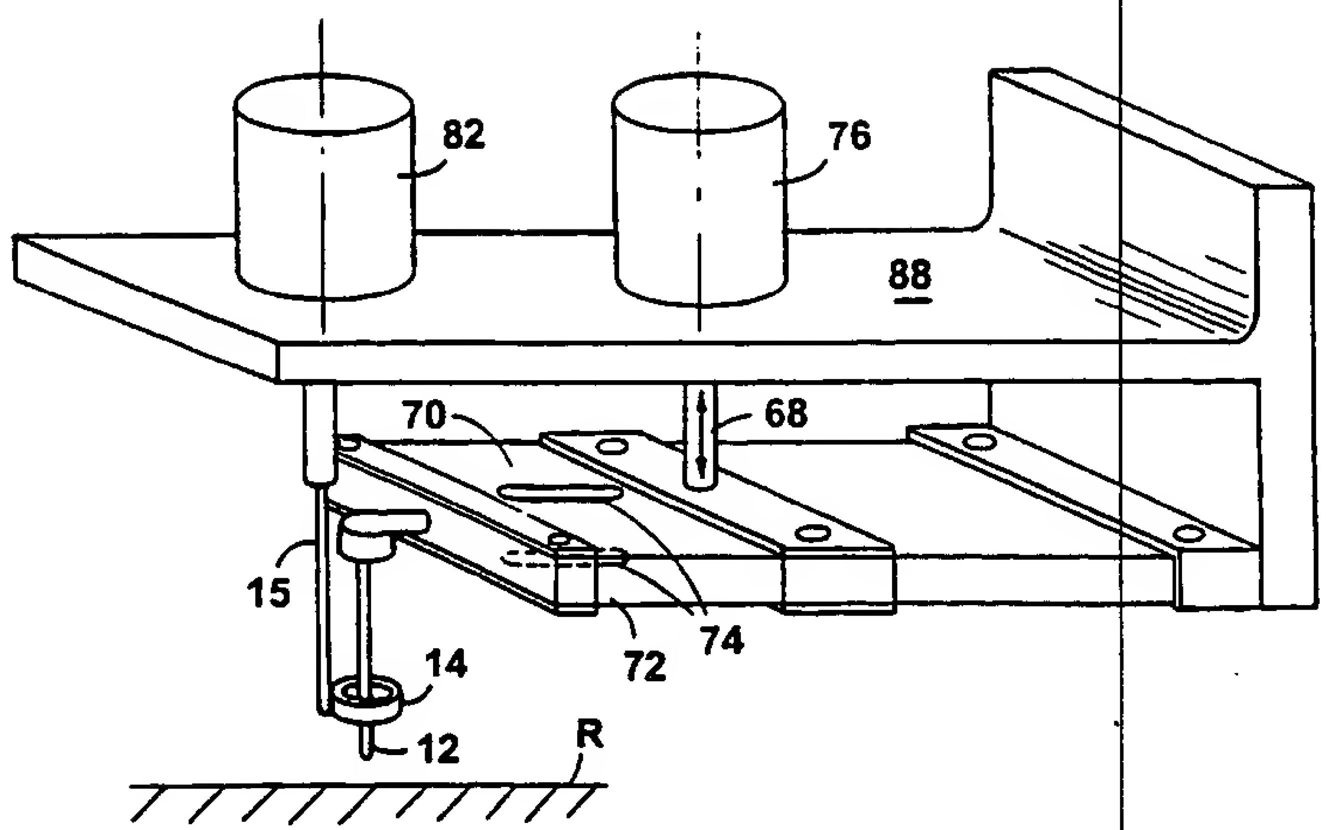


FIG. 23B

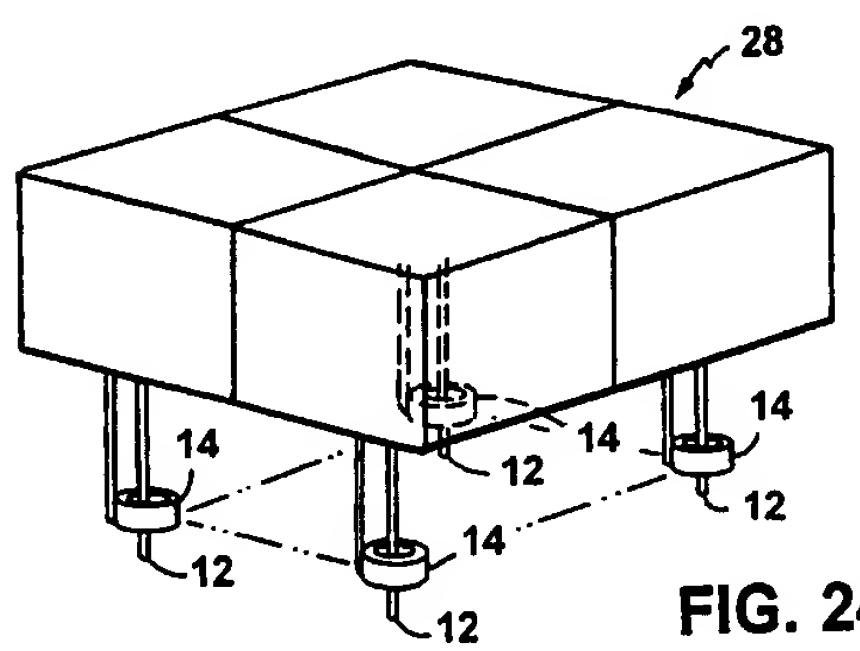


FIG. 24

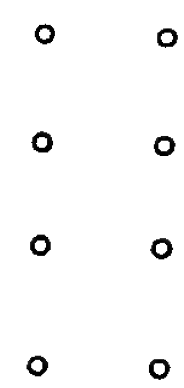


FIG. 24C

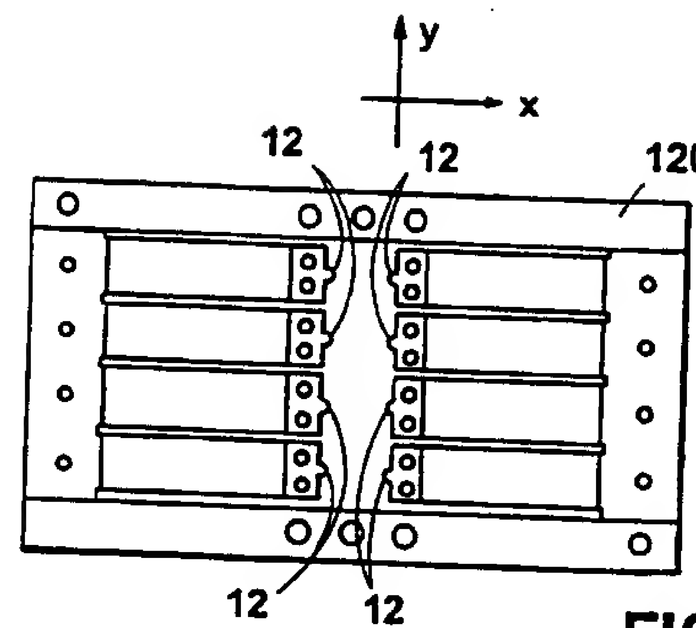


FIG. 24B

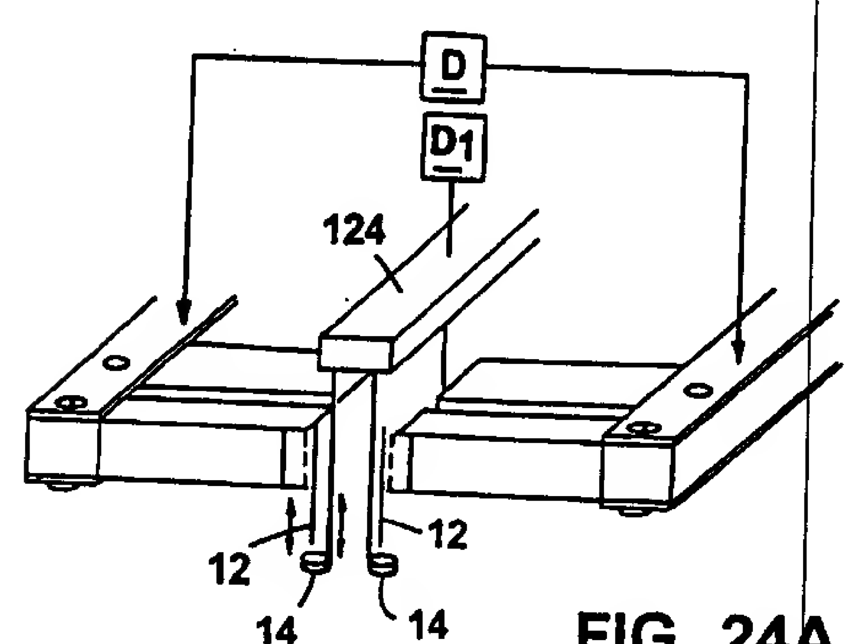


FIG. 24A

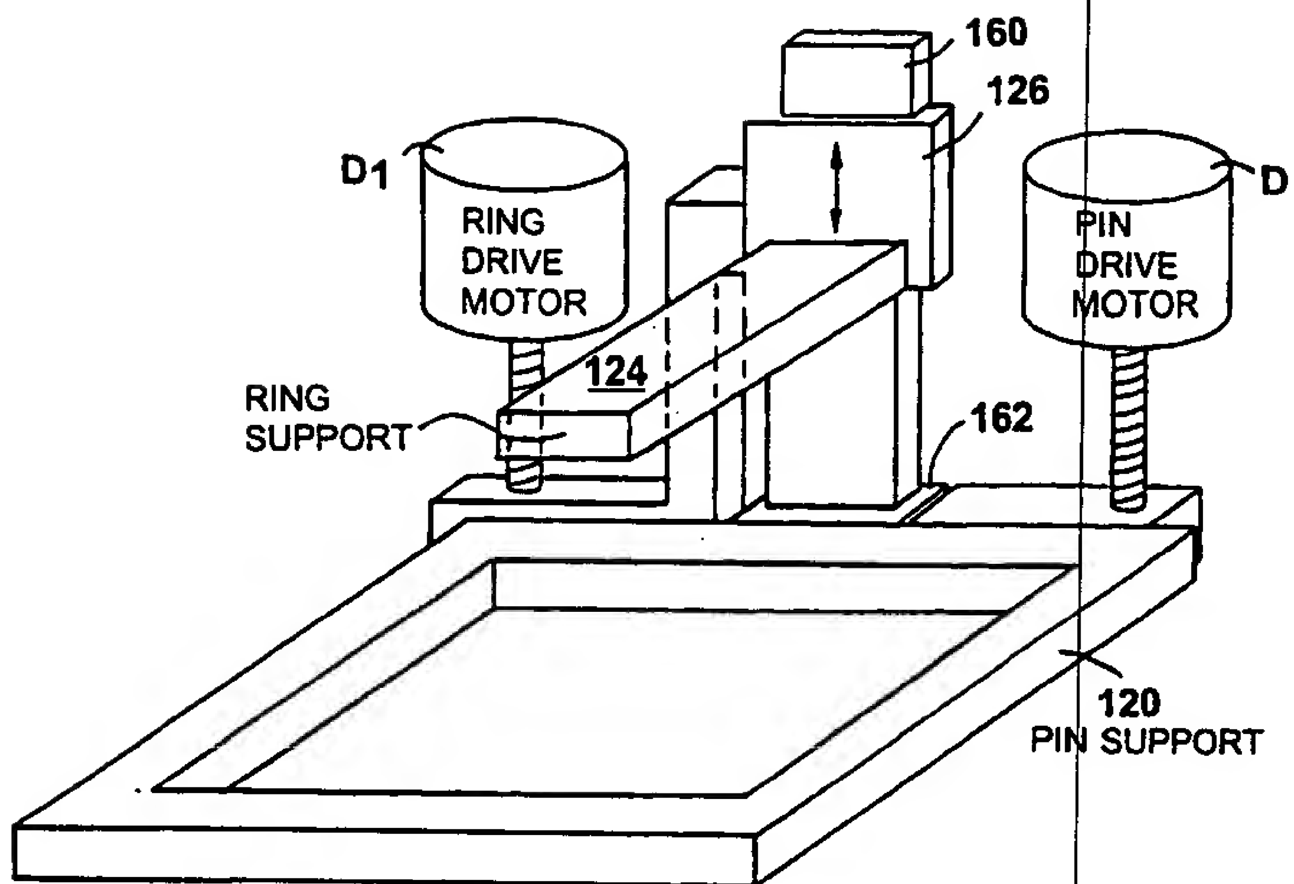

160
126
D1
RING
DRIVE
MOTOR
PIN
DRIVE
MOTOR
D
124
RING
SUPPORT
162
120
PIN SUPPORT

FIG. 24D

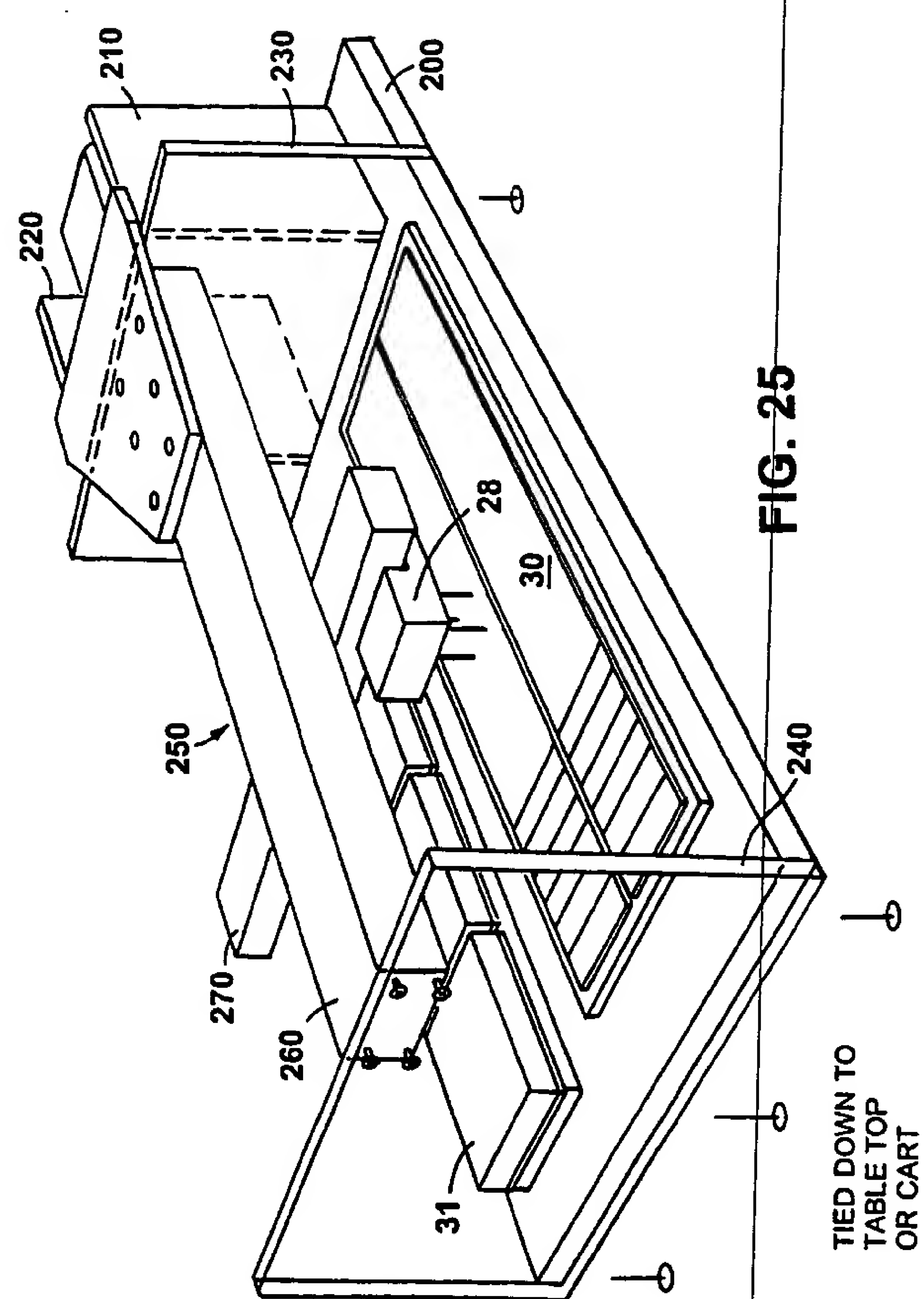

210
230
200
220
28
30
250
240
270
260
31
TIED DOWN TO TABLE TOP OR CART

FIG. 25

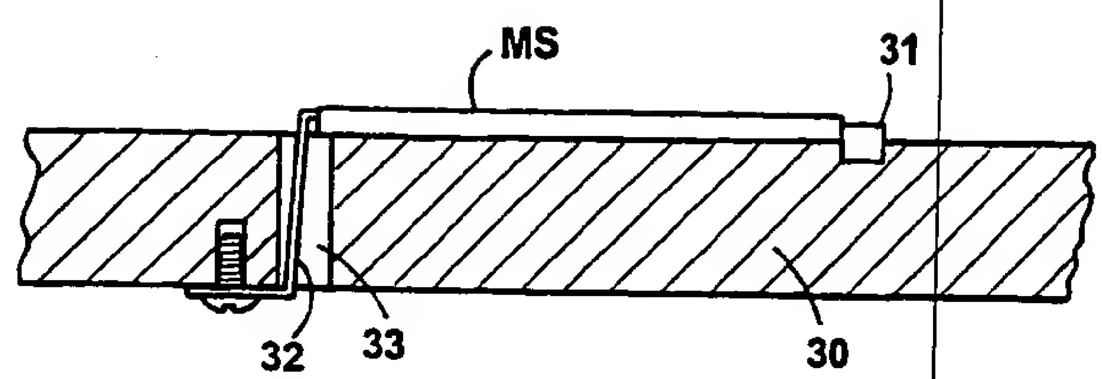


FIG. 25A

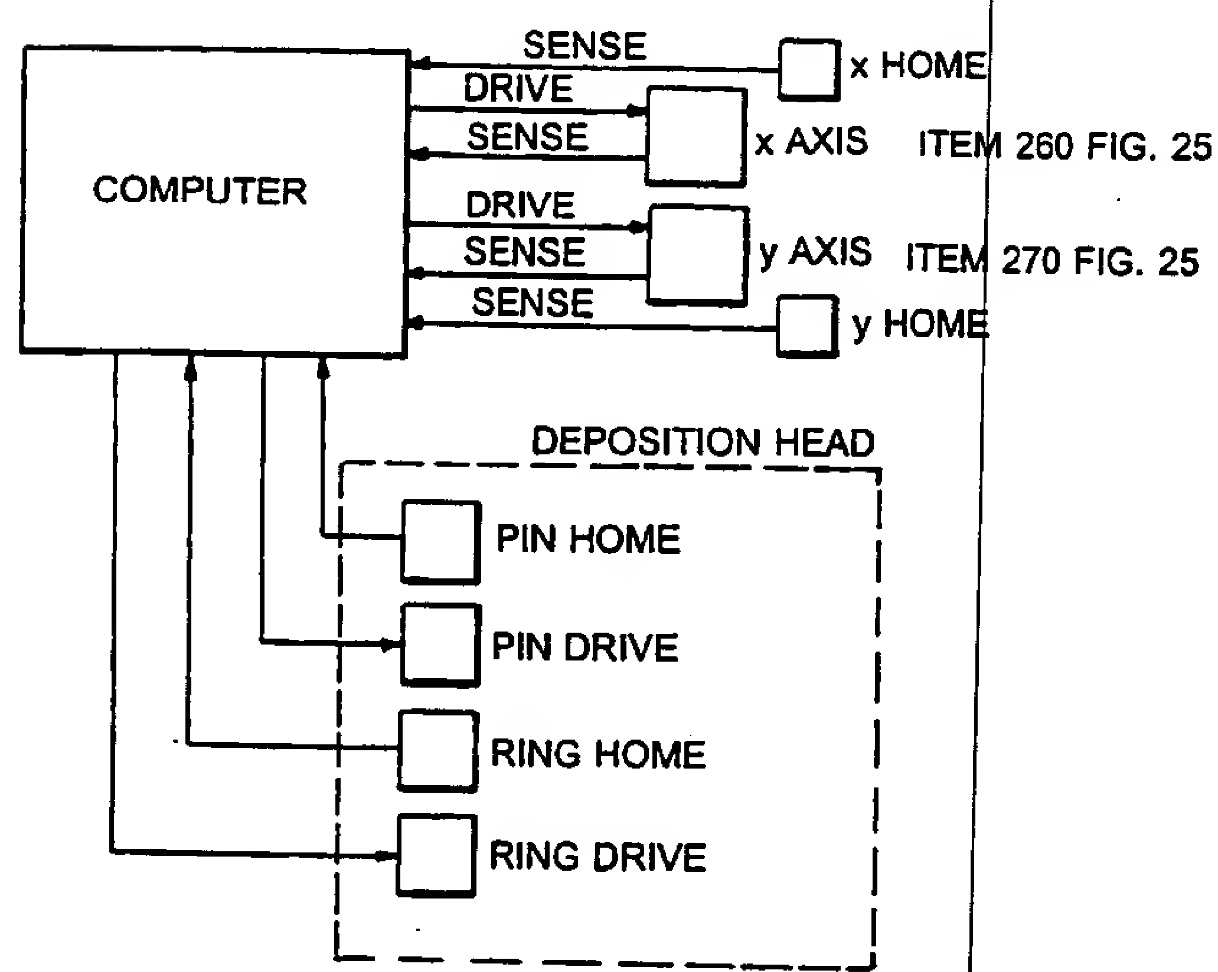


FIG. 26

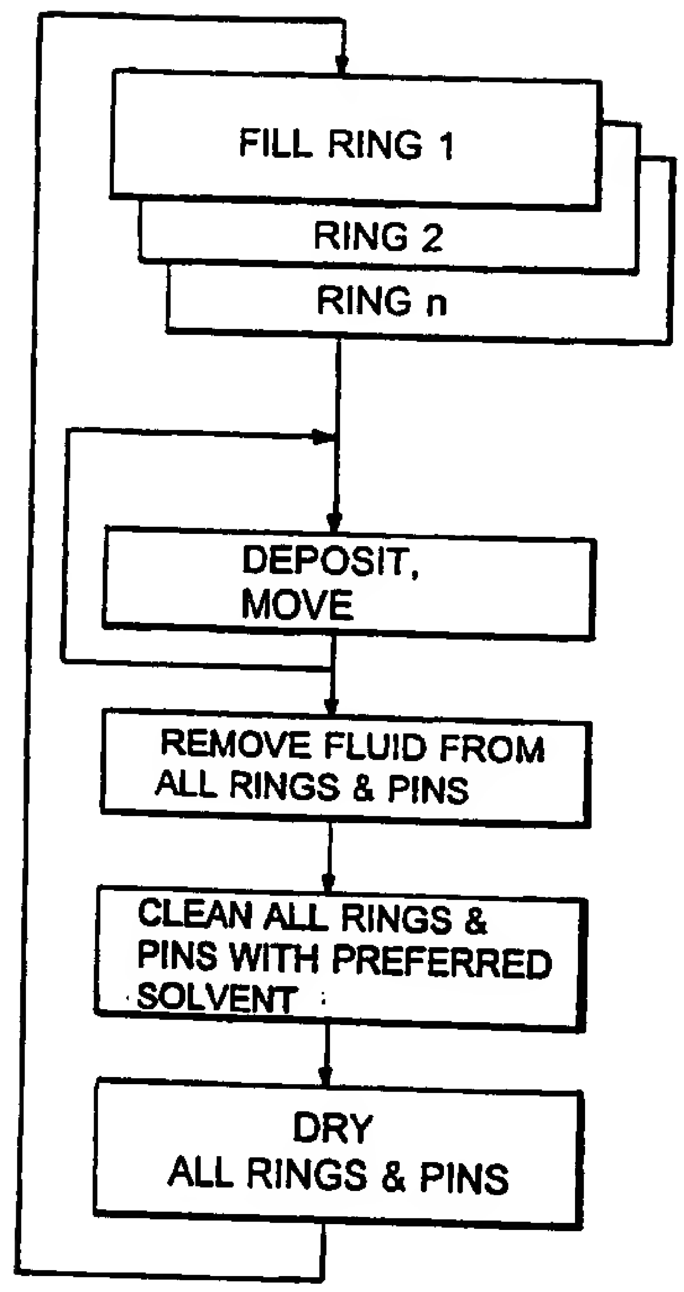

FILL RING 1
RING 2
RING n
DEPOSIT, MOVE
REMOVE FLUID FROM ALL RINGS & PINS
CLEAN ALL RINGS & PINS WITH PREFERRED SOLVENT
DRY ALL RINGS & PINS

FIG. 27